

Poor outlook for arms control

Something needs to be done to break the deadlock at the parallel bilateral negotiations at Geneva. Other governments could help. Even a summit meeting might be worth the risk.

HOPES that 1983 would bring substantial progress on arms control are fading fast. That is the obvious but depressing inference from what politicians in Moscow and Washington and negotiators in Geneva have been saying in the past few days. The latest disappointment is the swift and hostile response in the Soviet Union to the new negotiating position on strategic weapons announced last week by President Reagan, on the eve of his negotiator's departure for the resumption of the Strategic Arms Reduction Talks (START) at Geneva.

On the face of things, the United States seems to have conceded one important point on which the Soviet Union has for several months been pressing — that aircraft capable of delivering nuclear weapons should be counted in the strategic balance. It has also devised a workable way of making progress towards a more modest balance of strategic forces. The objective that there should be a reduction of the number of strategic warheads deployed of at least five per cent a year may be too modest to satisfy everybody, but anything is better than nothing. Moreover, the notion that these reductions should mostly be achieved by taking out of deployment more warheads installed on existing weapons than would be added on new missiles would have been less interesting to the Soviet Union than to the United States. But the principle of "build-down" is attractive, if only as a model for the future. It is a shame that it has been dismissed so quickly.

The Geneva negotiations on weapons of intermediate range (called INF) are messier. The Soviet Union has solidly resisted the notion that its deployment in the past five years of SS20 missiles within range of Europe is in any way provocative of the decision of the North Atlantic Treaty Organization (NATO) in 1979 that counterpoising cruise and Pershing II missiles should be deployed in Europe (from the end of this year). That is perverse. While the SS20s do not fall within the definition of what constitutes a strategic missile for the purposes of the unratified Salt II treaty (they cannot reach the United States), their appearance on the European scene was plainly an event of great strategic importance.

The Soviet refrain throughout the INF talks has, however, been that if there is to be an agreement on the numbers of missiles deployed, the British and French nuclear forces must be counted, which is at once reasonable and unreasonable. On one (Soviet) view, the SS20s are merely a counterpoise for the two non-American nuclear forces in Western Europe; on another (Anglo-French), European nuclear arms are strictly strategic in the sense that this is how the governments which operate them define their purpose. The British force (operationally controlled by NATO) is increasingly intended as an assurance that even if the United States commitment to Western Europe becomes shaky, there will be some kind of a nuclear deterrent left. The French force is what it has always been since the 1950s, a way of keeping unwanted visitors out of France. Especially because the two forces appear to have been counted implicitly as strategic in the Salt II agreement, the Soviet Union must know that the United States could not concede this point even if it were constitutionally able to do so. The moral for all concerned is, however, that the START and INF talks must sooner rather than later be thrown together. The attempt to distinguish between strategic and other kinds of nuclear weapons within the tiny compass of Europe makes less and less sense as time goes on.

With both negotiations at Geneva on such shaky ground, however, may it not be too much to hope that this frail forum will survive the deployment of the cruise and Pershing missiles in Western Europe? Already there have been hints that the Soviet Union may withdraw from the negotiations as a mark of its displeasure, while frustration with the Geneva process has some of the government's advisers in Washington complaining that by being apparently the only source of suggestions as to what kind of treaty might be signed, the United States is "negotiating with itself". The danger now is that one side or the other will allow pride to get the better of its judgement. Mercifully, there are influences working the other way. The side responsible for bringing to a halt the only substantial negotiations on arms control now under way will invite not merely displeasure in the public prints but trouble at the next review conference of the Non-Proliferation Treaty, now less than two years off, when the other signatories will be rightly looking to the superpowers for promised progress towards "effective measures in the direction of nuclear disarmament". Moreover, the issue is not simply which superpower is the least unpopular in the international community: the weapons on which their power rests are too dangerous for that.

What in the circumstances should be done? With all that has happened and not happened in the past few months, and with the emerging need for some kind of formula that would allow the two superpower governments to set off in a new direction without loss of face, nothing less than a personal meeting of the two political leaders (Messrs Andropov and Reagan) will now suffice. Such a meeting could agree that the two sets of talks at Geneva could be profitably combined, implicitly blaming the failures of the past two years on the agenda. It would also be possible to reaffirm the understanding of the two sides at the outset of the Geneva talks, now honoured only in the breach, that negotiations would be conducted in private, not by means of television broadcasts. A summit meeting, however hackneyed the formula, would also allow the two sides to end their public slanging-match about the destruction of the South Korean civil aircraft over Sakhalin. (The leak by the US intelligence services to the *New York Times* last Friday agreeing that the Soviet military thought they were shooting down a hostile military reconnaissance plane does not excuse the Soviet Union from the charge of incompetence but does mean that a great deal of the rhetoric in the past few weeks has been misplaced.) But may not summit meetings make things worse? (The Kennedy-Khrushchev meeting in Vienna in 1962 was after all followed within a year by the Cuban missile crisis.) One measure of the parlous state of affairs now reached is that the risk may be worth taking.

Other governments than those of the superpowers have a part to play. While the Geneva missile talks are too important to be dropped, there is an urgent need to blow away the dust from other almost forgotten negotiations — the comprehensive test-ban treaty aborted in 1980, a sensible treaty on chemical weapons (where again the Soviet Union has disappointed by failing to respond to proposals put up in the Geneva Committee on Disarmament) and even the notion that something might be done to make central Europe a less dangerous place. The best hope is that next year's meeting of the European Security Conference will provide a forum for agreement on some practical steps. □



Animal rights nonsense

The animal rights movement, if unchallenged, may blunt proper concern for animal welfare.

THOSE who espouse "animal rights" at least have consistency going for them. Attempts to reduce their arguments to absurdity generally result in that peculiarly dizzying sensation well known to political commentators and accepted by them as a sort of occupational hazard: one day's exaggeration is the next day's reality. Political satirists first succumbed to the affliction in the 1950s when one columnist, distraught at the way US presidential candidates were being sold to the public, wrote in a burst of fertile imagination that, the next thing you know, candidates will be hiring Madison Avenue advertising agencies. Satirists have since learned to avoid any words resembling "the next thing you know"; fleetness of foot now rivals nimbleness of mind in the satirist's job description; copy must be raced from the typewriter to the printer before reality overtakes satirical imagination.

Sensible people who would resist the claims of advocates of animal rights now suffer from a similar affliction. The trouble began when the animal rightists thought it necessary to develop a coherent philosophical basis for their beliefs. They can rarely today be faulted for having failed to think through the consequences of the premise that animals, no less than humans, are endowed with certain inalienable rights. Detractors who think their case is won with an elementary *reductio ad absurdum* such as "What if an animal must be sacrificed to produce a life-saving serum for a human?" are in for a quick lesson in the philosophical consistency of the animal rightists, who will coolly answer that a human life is not worth any more than an animal's, and that humans have no right to volunteer an animal for such a sacrifice. What if the human in question were your daughter, the detractors press? Even so, the rightists respond, humans can volunteer to help humans, but animals cannot be forced to sacrifice their rights for a cause that does not aid them.

Animal rightists are likewise consistently opposed to animal agriculture (an exploitation of animals) and are especially alarmed at the notion of breeding new strains of domesticated animals particularly suited to farming. And they explain the behaviour of animals in the wild, behaviour which undeniably includes the denial of the rights of other animals quite directly by eating them, by appeal to a concept of the fundamental nature of the animals in question, the "pigness" of the pig, as one has eloquently put it. Whether humans have a right to inflict a denial of the rights of helpless bacteria through such actions as scratching or noseblowing is finessed by appeal to a utilitarian concept of the capacity of various species to experience pain. At last, the *reductio ad absurdum* takes hold. They have been caught. But there is little satisfaction to be gained in skewering the animal rightists on their failure to stand up for bacteria.

Returning to the rightists' premise, however, can be made to yield to more direct hits. If animals have rights (the premise) then surely, like all who enjoy rights in a free society, they have responsibilities as well. Chief among those responsibilities is obedience to the laws of the society in which they live. To this end, a member of *Nature's* staff has conducted a modest experiment in animal responsibility. The results were not encouraging. One subject, a two-year-old sable and white collie, even when made aware of the penalties it faced, would sit on command on only two out of five tries. No progress was made, either, in convincing three chickens to adhere to even the most liberal of norms of order; they have consistently refused to lay eggs in a designated area. They failed totally, too, to sit on command. Voting is another responsibility of those in a free society; yet, considering the collie again, the ballot choices apparently would have to be limited to questions of food, elimination and copulation. And, as the Supreme Court has ruled, freedom of speech does not imply the right to bark at 2 a.m.

Serious obstacles also arise in attempting to transfer some accepted human rights to the animal world. The right to bear arms, anomalously still regarded as an inalienable right of US

citizens, is, to be sure, readily adapted as the right to arm bears. But, for example, several recent ethical studies have established the right of patients to refuse life-sustaining treatment. Domestic animals, routinely subjected to veterinary care, possibly against their will, are denied the chance to exercise this right.

Animal rights as a premise leads to some remarkable consequences, all the more remarkable for their affirmation by animal rightists. Yet the most direct consequence of the animal rights campaign is to give researchers an excuse to dismiss anyone who criticizes the use or treatment of animals in research as belonging to the same fringe that from time to time delivers to journals such as this proofs not only that special relativity is wrong but that the Central Intelligence Agency is engaged in a conspiracy to keep the truth from getting out. Lost in the fray is the voice of reason from the middle ground that bothers to make a distinction between animal rights and animal welfare. □

Triumph for unreason

The research community should be alert to the dangers of postponing a trial in genetic engineering.

THIS has been a sad week for rationality. A report elsewhere (page 564) records the decision of the University of California at Berkeley to postpone until next year its planned field trial of the usefulness of a strain of engineered bacteria for preventing frost damage in plants. Nobody will pretend that the decision is a major setback for agriculture in the United States. But it is a serious matter that Berkeley's decision will bring comfort to a small band of zealots who deserve none of it, Mr Jeremy Rifkin (see picture) among them.



Mr Rifkin is best known as the man who organized pandemonium at a meeting of the National Academy of Sciences arranged to provide an intelligent public discussion of the problems of genetic manipulation. Although the author of a book called *Entropy*, Mr Rifkin is not a scientist — as a casual inspection of that unfortunate document will show. (Maxwell's neat invention of his demons is as "a reflection of the hard-headed refusal of the scientific community to accept the full implications of what the Entropy Law means for science, philosophy and life on this planet", while Boltzmann's invention of statistical mechanics is a "remarkable sleight of hand designed to accommodate the second law [of thermodynamics] while undermining its clout".) Rifkin's declared purpose is to proclaim some version of the familiar message "The end of the world is nigh!"

It is customary to believe that people such as Rifkin should be ignored, eventually to be caught out by their own errors. That unfortunately is no longer the case. The postponement of the Berkeley experiment, even if brought about by the university's wish to avoid a legal action, is tantamount to an invitation to other Rifkins (or perhaps even the same one) to slap writs on other pieces of research they happen to dislike. Berkeley, in everybody's interests, should have stood up to him. □

US national laboratories

Government asks at last for changes

Washington

SOMETHING is, after all, to be done about the national laboratories. Heads of research for nine federal agencies are meeting this week to plan their response to a presidential call for sweeping changes in the way the government runs its 755 federal laboratories — which together spend some \$15,000 million a year, nearly a third of the federal science budget. The meeting is the first of a new committee chaired by the White House Office of Science and Technology Policy (OSTP) which has been given until July 1984 to begin to implement the recommendations of a report on the laboratories published earlier this year by a panel led by David Packard of the Hewlett-Packard Company.

When the White House Science Council issued the much-delayed Packard report in July it was greeted by a chorus of yawns. After a year of work, Packard and his colleagues produced a skimpy document which repeated the findings of a score of similar reports over the past two decades (see *Nature* 21 July, p.199). Like its predecessors, the Packard report complained that government departments meddle too much in the detailed management of their laboratories, that the missions of individual laboratories are often fuzzy and that government scientists, hamstrung by civil service regulations, are paid too little.

Soon, however, the yawns may have to be stifled. The big difference between the Packard report and its precursors is that it appears to have won the immediate endorsement of the President. In August, President Reagan ordered the heads of all government departments to cooperate with the Office of Management and Budget (OMB) and OSTP in drafting a plan to implement the report. Packard, a personal friend of the President, intends to ensure that at least some of the report's recommendations stick.

The inter-agency group will have an uphill struggle. Some recommendations, such as appointing laboratory directors on fixed-term contracts and improving relations between federal laboratories and the universities, are straightforward. Others are certain to face opposition from Congress and from the very government departments called on to implement them. These are the main difficulties:

Pay and promotion: Packard wants the pay of federally employed scientists raised substantially to compete with universities and industry. At present, government-operated laboratories are hampered by a civil service salary ceiling that makes it impossible to match the salaries offered elsewhere to very senior scientists. For exam-

ple, the top salary that the National Institutes of Health (NIH) can offer a physician — \$73,800 — compares badly with the salaries of between \$90,000 and \$120,000 commonly earned by department chairmen and full professors in medical schools. But other laboratories suffer too: last year the Naval Research Laboratory could offer only \$58,000 when advertising for a technical director to supervise a staff of several thousands and a budget of several hundred million dollars. Entry level salaries also lag. Federal laboratories looking for a newly graduated engineer in a "hot" field such as electronics can pay only \$22,000 compared with an industry average of \$27,000.

Civil service rules complicate promotions as well. Advancement through pay grades is linked to administrative responsibilities, making it difficult for laboratories to reward staff for their scientific performance alone. And a merit pay system introduced by the 1978 Civil Service Reform Act links bonuses to performance that is measured over a single year at a time.

To improve matters at all effectively will mean persuading Congress to pass legislation that will increase federal spending and asking the civil service unions to agree that some of their members should receive higher pay while others continue to lose

London merger

THE University of London last week was mildly pleased with the first response of the British University Grants Committee (UGC) to its proposals for a radical internal restructuring. In a letter from the new chairman of the committee, Sir Peter Swinnerton-Dyer, the university was told that its plans for amalgamating Bedford and Royal Holloway colleges on the latter's site at Egham, Surrey, will be given as fair a wind as UGC can manage.

The letter also says that UGC "warmly approves" the plan to build extra student accommodation at the site and even raises the question whether enough students are being catered for. (UGC has agreed that the new college should mortgage part of its site for construction money.)

The only fly in the ointment is that UGC wants further discussion on the intended student population at the new college in the light of the British Government's projection of falling student demand in the 1990s and the likelihood that places in science and technology will be favoured over those in the arts and humanities. A further difficulty for the merged college is that its size may be reduced below the 3,000 students now planned. □

their jobs. Administrators within departments that run federal laboratories can also be expected to complain if the pay of scientists who are nominally their juniors is allowed to overtake their own.

Government oversight: Packard wants to end "micromanagement" — the habitual tendency of departments to interfere in detailed management of the laboratories. The departments agree in principle but balk at giving up too much control. The Department of Energy (DoE) is looking for ways to cut paperwork (Oak Ridge claims it must file 700 progress reports a year). It is unlikely, however, that DoE wants to establish a genuinely arms-length relationship with its laboratories. The future of the department itself remains in doubt and its tight control of the laboratories is a weapon it will not willingly surrender.

On one micromanagement issue, DoE and OSTP have already squared up for a fight. The Packard report, complaining that laboratory directors have too little say in selecting their own priorities, wants between 5 and 10 per cent of the laboratories' budget to be spent at the discretion of the directors. DoE officials maintain that in the case of the major energy and weapons laboratories it would be foolhardy to give directors control of such large amounts of money. So far, DoE is unwilling to consider doing much more than setting aside between 1 and 2 per cent of the laboratories' budgets as "seed money" for exploratory research. OMB and the appropriations committees in Congress are likely to agree with DoE's position.

Defining missions: According to Packard, many federal laboratories are uncertain about what their proper mission is, or continue to perform missions that are no longer useful. Parent departments see this aspect of the report as a golden opportunity to shake up or even close some of the 700 or so laboratories which employ fewer than 500 professional staff. But most of the money for federal laboratories — nearly 70 per cent — is consumed by the 50 institutions with more than 500 staff.

Packard and OSTP do not want the major facilities to be let off the hook just because their size makes it difficult for them to change direction or, indeed, close down. OSTP suspects, for example, that many of the laboratories established by the National Aeronautics and Space Administration (NASA) have had little purpose since the end of the Apollo programme. OSTP would also like to use the Packard exercise to force major changes on some DoE laboratories. At his press conference in July, Packard said the Argonne and Brookhaven laboratories needed to think again about their roles.

Agency representatives on the new committee, meeting this week, can be expected to applaud the Packard report's call for increased efficiency and better management. They may well shudder at the prospect of a major political fight over the future of their biggest laboratories.

Peter David

Genetic engineering

Frost damage trial halted

Washington

FIELD testing of bacteria genetically engineered to reduce frost damage to plants has been set back. The University of California at Berkeley announced last week that an experiment involving the release of genetically-engineered bacteria into the environment (*Nature* 22 September, p.262) would be postponed until next year. The announcement came after Jeremy Rifkin, a Washington-based activist who opposes genetic engineering, threatened to seek a temporary restraining order against the university to halt the experiment. Rifkin had earlier filed suit against the National Institutes of Health (NIH), claiming that the NIH Recombinant DNA Advisory Committee failed to consider all the risks of the experiment, which it approved last spring.

The experiment, which would have been the first in which genetically engineered organisms are released deliberately to the environment, is designed to test the ability of the genetically altered bacteria to protect plants against frost damage. Normal strains of the bacteria, *Pseudomonas syringae*, excrete a protein that serves as a nucleation site for ice formation on the plant at temperatures slightly below freezing. In the genetically engineered strain, synthesis of this protein has been inactivated.

Dr Steven Lindow, principal investigator for the experiment along with Dr Kickolas Panopoulos, said last week that only two weeks of favourable weather remain this fall at the field site in Tulelake in northern California. "We're in a no-win situation", he said. A ruling on the motion for a restraining order could not come in time for the experiment to be carried out before heavy frost sets in. The altered bacteria are expected to provide protection down to 23°F, but temperatures below that are expected soon.

Lindow said that the university's legal counsel was "quite willing to defend the experiment", but that practical considerations dictated postponement. The researchers hope that Rifkin's suit against NIH will be resolved by next spring. The decision to postpone the experiments keeps the university from becoming directly involved in the legal action.

Lindow and Panopoulos have for three years conducted field trials of chemically-mutated bacteria similarly deficient in the ice-nucleation protein. Those tests demonstrated that introduction of a large population of protein-deficient bacteria can confer frost protection by displacing the native bacterial population. They also showed, Lindow says, that the experiments are basically safe; the altered strain lived only about three months before the native bacteria reasserted themselves, and did not spread to nearby plants. According to

Lindow, individual bacteria deficient in the ice-nucleation protein occur naturally in the environment as a result of mutations induced by ultraviolet light. He adds that the genetically engineered version is likely to be safer than the chemically mutated version because the process is more specific, deleting only the specific gene and not altering others. No permission was required to field-test the chemically

mutated bacteria.

Rifkin meanwhile has obtained depositions from several more scientists opposing the experiments. Peter Raven, director of the Missouri Botanical Garden and professor of botany at Washington University in St Louis — and a member of the National Academy of Sciences — argues in a deposition that "the environmental impact of these bacteria on natural plant populations might be severe". He argues that the experiments should not go forward without further review of their ecological consequences. **Stephen Budiansky**

Swiss science policy

The law enters the arena

Berne

SWISS science — normally a rather private (and successful) affair — stepped cautiously into the political arena last week. The cause: the passage through the federal parliament of Switzerland's first "law for research", which requires the federal government to define a policy for science. On the surface, the law is anodyne enough, but it has a hidden significance: scientists in Switzerland are submitting themselves to political will in order to ensure themselves of financial support, while politicians are becoming aware that science may provide a key to economic recovery.

The deal may work: at least, in the short

Research and development spending per head

Switzerland	£178
West Germany	£137
USA	£123
Sweden	£113
Netherlands	£102
France	£93
Japan	£86

Values are in pounds sterling per inhabitant for 1979, from Schweizerischer Handels- und Industrie-Verein, research and development report 1980.

term it may work for the scientists. For also being debated last week was the federal budget for science and education (largely a matter of grants to the regionally-controlled universities), and it seems this budget will show a modest increase on last year. This would be a boon. The (Swiss) National Science Foundation (NSF), a nominally private institution which supports most basic science (and humanities) in Switzerland, suffered a 10 per cent real cut in funds in 1980, and has barely held constant since. Next year (1984), at least a 2 per cent real increase in NSF funds seems certain, and a group of parliamentarians (the committee on science and education) is pushing for a 13 per cent increase to SF 174 million (£56 million), the sum actually requested by the foundation as a bargaining position earlier this year. The final figure will be decided in December and although it may not reach 13 per cent, it should be good for NSF.

On the other hand, this new-found political interest in science should result in

a politically-determined policy: in other words, some fields unattractive to politicians should eventually feel the pinch. However, the federal government still has no minister of science (or of education, which is controlled, kindergarten to university, by the cantons), and its policy will still largely be influenced by the advice it receives from the Swiss science council (a body of the grand old men of science). This body also liaises with NSF and with the other principal institution in Swiss science policy, the office for science and education (under the ministry of the interior). These three will thus effectively determine Swiss science policy as at present. However, in the past this job may have been easier than it will be now.

Before the 1980 shock, scientists in Switzerland more or less got what they asked for. As a result, Swiss spending on research and development rose to some SF 3,000 million — £1,000 million — of which three quarters was spent by private industry, a sum which made it the highest national research and development spend per head of population in the world (see table). Swiss scientists, however, claimed this was only what they needed. Now, however, they are not likely to get exactly what they need (the 13 per cent on the NSF budget). So the National Science Foundation (in consultation with the other bodies) has made choices, and the politicians have been impressed. This has been the political trade-off. Certain fields will "level off", a code for constant funding in current Swiss francs, or in other words a real fall according to the current rate of inflation (around 2.5 per cent).

Fields threatened with "levelling-off" in the NSF funding request are many, but tend to cover the more outmoded aspects of modern sciences, such as "classical aspects" of algebra, geometry and analysis in mathematics, homologous series in chemistry or "conventional" research in cell biology. Singled out for support are "centres of excellence" in a somewhat shorter list of subjects. All good stuff for the political paymasters, no doubt, and a chance, perhaps, for NSF to do some useful weeding. **Robert Walgate**

US science budget

Congress outdoes President

Washington

PRESIDENT Reagan is learning the hard way that even when presidents believe they are being generous, Congress is likely to disagree. In his 1984 budget request last April, the President proposed major increases for most areas of scientific research. Total federal research and development was promised an increase of 17 per cent over 1983 and basic research was to increase by 10 per cent, including an 18 per cent increase for the National Science Foundation. After eight months of congressional wrangling, it seems that Congress has been even more generous to science, although its research priorities are rather different.

Department of Defense: The President's defence budget, the most controversial part of his 1984 spending plan, is bound to be reduced when it is finally debated on the floor of Congress. The defence subcommittee of the Senate Appropriations Committee voted last week for a total budget of \$250,000 million — some \$10,000 million less than the President wanted. The Pentagon's basic research programme, much of which finances work in the universities, suffered a last-minute setback when the House Appropriations Committee sliced \$30 million from the President's request of \$850 million. The outcome will depend on a House-Senate conference.

National Aeronautics and Space Administration: NASA's appropriations bill has been passed and the agency's 1984 operating plan is almost identical with President Reagan's request for space science. Physics and astronomy will get \$567 million instead of the \$514 million the President requested, but most of the increase will pay for cost increases on the Space Telescope.

National Institutes of Health: NIH are operating under the continuing resolution, which expires on 15 November. However, the House of Representatives has already passed a generous appropriations bill and the Senate is completing action on a virtually identical measure. Both give NIH \$4,460 million, give or take a million — a substantial increase, as expected, over the President's request. Congress's appropriation will maintain the number of extramural grants at 5,000, while restoring cuts that had been made in other programmes to meet this goal. The congressional action also will do away with the administration proposal to cut indirect costs, or overheads, on grants to 90 per cent of current levels and to renegotiate direct costs on existing grants downwards by 8–12 per cent.

Congress's stalemated attempts to pass an authorization bill for NIH do not affect their research activities, which operate under standing legislative authority.

National Science Foundation: The President got everything he asked for from Con-

gress, together with a lot that he did not want in the form of science education programmes. The total increase for the NSF budget this year comes to 21 per cent — a total of \$1,320 million. Research and related activities receive \$1,242 million, including \$15 million earmarked for high technology instrumentation and \$5 million to be set aside for instrumentation at non-PhD-granting universities. It is up to NSF itself to allocate the total which it is sure to follow the plan in the President's budget, which emphasizes mathematics and physical sciences. The President also calls for NSF to award, within its regular grant programme, \$180 million for instrumentation.

In the battle to see who could appear more concerned about the supposedly desperate state of American mathematics and science education, the bidding in Congress reached \$70 million for NSF's educational activities. In addition to the teacher training programme and graduate fellowships that the President asked for (\$30 million), Congress's appropriation includes funds for curriculum development, something that the Reagan Administration has been philosophically opposed to.

Department of Energy: In basic energy sciences research and development — which includes high-energy and nuclear physics — the President also received everything he asked for, but not in the way he wanted. The congressional appropriation cuts the administration request for the planned National Center for Advanced Materials (NCAM) at Lawrence Berkeley Laboratory from \$25 million to a mere \$3 million, the result of criticism that the proposal had not been properly reviewed and of some pork-barrel entrepreneurship on the floor of the House by Catholic Univer-

sity and Columbia University. The Stanford Linear Collider, Burton Richter's ingenious electron-proton accelerator that may yet be on line before the European Organization for Nuclear Research (CERN)'s LEP, was granted 80 per cent of the administration's \$40 million request. Construction will begin this year.

The administration's attempt to cut back on solar and other non-nuclear energy research and development met with stiff opposition. Solar, which the administration wanted slashed in half, was given \$174 million, down only slightly from 1983 levels. Geothermal, fossil and conservation — all slated for a similar cut — will all be maintained at close to 1983 levels. Nuclear fission research, which was to receive an increase in the administration's plan, however, was cut by over one-third from the 1983 level of \$746 million.

Department of Agriculture: Although a final appropriation has not been passed for USDA yet, the House and Senate have approved separate, somewhat different versions, that at least indicate what the final appropriation will be. The major difference between the two bills is in the competitive grants programme, the only source of agricultural research support open to non-land-grant universities. In the House, Representative Jamie Whitten (Democrat, Mississippi), a perennial foe of the programme, succeeded in cutting not only the small \$4 million increase proposed by the administration, but a further \$7 million from the heart of the programme as well — leaving a mere \$10 million. The Senate went along with the administration request and voted \$21 million.

The House would hold the two major USDA research programmes roughly to 1983 levels (\$233 million for the formula funds that support research at land-grant universities, \$470 million for the in-house Agricultural Research Service); the Senate would increase both by a few per cent. □

US risk assessment

Congress looks to Academy

Washington

UNDAUNTED by a string of previous failures, Congress is considering new legislation designed to improve the way federal agencies regulate hazardous substances. A bill emerging last week from the House of Representatives subcommittee on science research and technology would have the National Academy of Sciences create an expert board to review the scientific basis of regulations derived from risk analysis techniques. Its recommendations would not be binding but agencies which ignored them would be required to give a detailed public explanation of their decision.

The bill, proposed by New York Republican David Martin, is a watered-down version of a controversial measure first proposed three years ago to create a national science council to resolve scien-

tific disputes arising from regulatory decisions. Under that proposal, the council's decisions would have been binding and any member of the public would have been able to refer issues to it. Martin's board, however, would discuss individual regulatory decisions only when asked by the federal government and the scope of its review would be narrower.

Unlike the earlier proposal, Martin's bill has a good chance of becoming law. He claims to have cleared the proposals with the National Academy and the White House Office of Science and Technology Policy. Moreover, during the subcommittee deliberations last week, the bill was incorporated in another bill, the Risk Research and Demonstration Bill, which already enjoys widespread support.

Peter David

Star wars

New hope for US space platform

Washington

PRESIDENT Reagan's controversial suggestion last March that the United States should build a futuristic system of defence against nuclear attack may turn out to have given the National Aeronautics and Space Administration (NASA) a winning argument in its campaign to build a manned space station by 1991. Leaks from a panel set up by the White House to examine the President's "star wars" concept suggest that its report, due to be completed next month, will say that development of a space station might be essential to test the precise pointing systems and mirrors required by a space-based defence.

Without the happy coincidence of the President's "star wars" initiative, NASA's chances of winning approval for the space station were distinctly shaky (see *Nature* 18 August, p.574). The agency's administrator, James Beggs, maintains that a space station is the logical successor to the shuttle and the best way of maintaining US leadership in space. But the National Academy's space science board declared last month that there was no immediate scientific justification for a manned station, and many in Congress and the

Air Force is anxious to see the size of the shuttle fleet and its spares increased. It also wants NASA to develop an extended mission orbiter, an upper stage and a tele-operated manoeuvre system for the shuttle.

The Air Force may be forced to change its priorities if the White House "star wars" panel, itself chaired by a former NASA administrator, James Fletcher, sings the praises of the space station. The Department of Defense is also coming under mounting pressure from its own advisers to adopt a more flexible attitude to its possible military uses. A report released last week by the Air Force Scientific

Advisory Board warned that while no existing missions required the use of a space station, future missions might. Potential new missions listed in the report include a space-based antimissile defence.

A change in the attitude of the Pentagon, combined with a desire to defuse the electoral challenge from John Glenn, may tip President Reagan's decision NASA's way. But identifying the space station too closely with the President's plans for a watertight defence against nuclear attack may jeopardize the space station's chances in Congress. Many congressmen who favour building a space station will withdraw their support if a vote for the station can be construed as a vote for building a nuclear defence that is widely regarded as unworkable.

Peter David

UK weapons tests

Search for damage mounted

THE National Radiological Protection Board (NRPB) has announced its detailed plans for a survey of cancer incidence and mortality among more than 12,000 civilians and ex-servicemen who participated in British nuclear weapons tests, including atmospheric explosions and other unspecified experiments, in Australasia between 1952 and 1958.

The study was commissioned by the Ministry of Defence after press reports earlier this year alleged that safety measures during the tests and in subsequent clean-up operations were inadequate. However, a review of the Australian tests carried out this year by the Australian Ionising Radiation Advisory Committee found no evidence of serious safety lapses, and the British Ministry of Defence has steadfastly maintained that, even by today's standards, safety measures were "sufficient to ensure that no test participant was exposed to any significant risk of injury or disease".

Last April, a preliminary study by Professor E. Knox of the University of Birmingham's Department of Social Medicine found unusually high levels of self-reported leukaemias and other neoplasms of the reticuloendothelial system among test veterans. However, Knox *et al.*'s conclusions depend entirely on the number of individuals involved in the test programme, and conflicting answers have been given to this question. The NRPB study will avoid the problem by comparing cancer mortality and morbidity in test personnel with data from a "control" group of servicemen who were also in the tropics at the time but not near the test locations.

NRPB's study protocol reveals a surprising ignorance about the identities of those involved. Some 12,000 names have already been assembled by the Ministry of Defence, but it is clear that the list is far from complete. According to Dr John Reissland, who heads the NRPB team, this is because military records are arranged ac-

cording to individuals' names, rather than by operations. Self-reported cancer cases will also be assessed by the board, but, in order to avoid bias, these will not be included in the main study. Mortality information and cancer registrations will be obtained from the National Health Service's Central Register, which records all cases of cancer diagnosed in Britain since 1945.

Mr David Alton, a Liberal Member of Parliament who has been pressing for a study on test veterans since early this year, is not happy that the commission has gone to NRPB. "NRPB is too closely identified with the government", Alton says, and the public will not be confident that the survey is unbiased. Alton will also be pressing in Parliament for changes in the law to allow test veterans to claim compensation.

Dr Reissland explained last week that the NRPB contract ensures that the Ministry of Defence will have no control over what findings are published, and that because the study will not require access to classified material, the ministry will not be able to exert even indirect control. A study of safety measures that will require privileged access is being kept separate for this reason. All findings will be published in full, but because tracing all those involved is likely to be time-consuming, results may not be available for at least two years.

Others have reservations about the scope of the NRPB study. Professor Joseph Rotblat, a radiologist who worked on the weapons programme, wants to see a similar study done on the incidence of cataract, which will not be considered by NRPB. In collaboration with Mr Anthony Bron, Rotblat has found among veterans of tests at Christmas Island, 25 self-reported cases of posterior subcapsular cataract, a rare type almost unknown in men of this age. Rotblat suggests that either accepted threshold levels of radiation for cataract formation are substantially in error or that dosage was higher than the ministry admits.

Tim Beardsley



Office of Management and Budget (OMB) believe an unmanned system could achieve similar goals for less money.

The weakest point in NASA's argument has until now been the frosty attitude of the Pentagon. Richard DeLauer, Under-Secretary of Defense for research and engineering, said last August that the Department of Defense could not identify a single military mission that could be better carried out by a space station than by the shuttle or an unmanned spacecraft. And senior Air Force officials continue to express concern that building a space station would distract NASA from further development of the shuttle.

According to Charles Cook, its deputy assistant secretary for space policy, the US

Australian veterinary science

Canberra's quandary on FMDV

Canberra

THE continuing dispute about foot and mouth disease virus (FMDV) is almost as rancorous as would be the row if the disease were ever found in Australian livestock.

A document released last month by the Animal and Veterinary Science Committee of the Australian Academy of Science has prompted the government to set up yet another committee of experts to ponder the fate of the \$A157 million Australian National Animal Health Laboratory (ANAH), now almost complete, at Geelong, Victoria (see *Nature* 303, 190; 1983). The maximum microbiological containment facility was built primarily for work with FMDV, but the government has now decided to ban for five years the importation of the virus, which strikes fear in the heart of the Australian Farmers Federation, now in the apparently paradoxical position of wanting ANAH but not FMDV (see *Nature* 303, 463; 1983).

The underlying dispute is whether live FMDV is necessary for the diagnosis of the disease in animals. The new document, entitled "A proposal for the establishment of a diagnostic capability for FMDV in Australia", was prepared by Professor Bede Morris, chairman of the academy's Animal and Veterinary Sciences Committee, but has not been considered and endorsed by the academy's council.

Professor Morris, who heads the anti-ANAH faction of the Australian National University in Canberra, proposes on-farm diagnosis based on epidemiology, clinical symptoms and, when required, host susceptibility tests in which a range of animals would be infected with putative virus in order to distinguish FMD from other vesicular disease. Since FMD is known to travel downwind for considerable distances, and infected animals, especially pigs, excrete large numbers of virus particles, the procedure is controversial, and also inconsistent with the view that the introduction of FMDV into ANAH is unacceptable. Professor Morris says a definite diagnosis would be made at the world FMDV reference centre at Pirbright (United Kingdom) but that action — slaughter and quarantine — should be taken on clinical symptoms alone. In these circumstances, which apply at present, it is difficult to rationalize on-farm differential diagnosis at all.

The Commonwealth Scientific and Research Organization (CSIRO) has responded with a commentary which contradicts the Morris document on several points. It says that Pirbright offers a typing and not a diagnostic service and quotes evidence that samples sent overseas may deteriorate *en route* and that the positive control offered by the use of live virus in the laboratory diagnosis is essential. (Professor Morris warns of the

"totemism" of laboratory tests.) CSIRO and Morris disagree about the availability and cost of diagnostic reagents for the 7 types and 60 subtypes of FMDV. Underlying the CSIRO argument is the fear that Australia could at some stage lose its FMD-free status in the international beef market on the basis of an unconfirmed diagnosis.

If the Morris proposal was intended, as seems likely, to sway the government into mothballing the laboratory, it seems to have been counterproductive. Uncertainty about the status of the document put the Minister of Science and Technology, Mr Barry Jones, in the invidious position of having to defend the proposal in the House of Representatives on 20 September. Hitherto, Mr Jones had had a sympathetic ear for the anti-ANAH group and his position is in part responsible for the antagonism between him and Dr Paul



Barry Jones, for the defence despite himself.

Wild, chairman of CSIRO, which he describes as "creative tension".

The new committee, under the chairmanship of Professor Frank Fenner (now retired from the Australian National University) is clearly conceived of as a final resolution of the FMDV problem. Its terms of reference leave no doubt that the government does not know what to do about the laboratory and that it is confused by the controversy among scientists. Should ANAH undertake research and diagnosis? Should the facility be operated at its maximum containment level at a cost of \$A8 million a year? Should ANAH make FMDV vaccine? Should the laboratory become the animal virus reference centre for South-East Asia? The government, no longer sure whom to believe, hopes the committee will tell it what to think, and quickly.

Vimala Sarma

Polish agriculture

Walesa award fuels problems

THE award of the Nobel Peace Prize to Lech Walesa has focused world attention not only on the controversial Solidarity leader but also on the proposed bishops' fund for the relief of Polish private farmers to which Mr Walesa has decided to donate his winnings.

This fund is a sensitive issue in Polish politics. It was first mooted early in 1982 by Catholic bishops in Western Europe and North America, who proposed to raise a sum of DM 5,000 million (£1,300 million) (25 per cent from the Church, 25 per cent from Western governments, 50 per cent from Western bankers) to finance low-interest loans to farmers, the establishment of a non-governmental self-managing agricultural supply and purchasing network, and the provision, in rural areas, of roads, food processing plants and essential social amenities. Polish private agriculture, during the Gierek era, had been considerably run down under a pricing system which grossly favoured state and collective farms; moreover, although the latter accounted for only 25 per cent of cultivated land, it received 64 per cent of all agricultural investment.

The Polish Government was clearly not happy about a project that was outside its control, but eventually agreed to participate in a joint working party. It then continued its delaying tactics, emphasizing that a legal framework must first be established to allow this unprecedented intervention by the Church in non-spiritual matters. Finally, it said that if the object of the exercise was really to rescue Polish agriculture, then part of the funding must go to the state sector to avoid the risk of misinterpretation.

The bishops explained that the Western sponsors would probably be unwilling to support the state sector, but the government continued to press the point. A further set of difficulties then emerged concerning the increased production and income which the scheme, if implemented, would generate. The bishops say that this must be used, at least in part, to repay the bankers and governments concerned; the Polish Government now demands that the capital thus generated must be reinvested in Poland. This, of course, fundamentally changes the structure of the scheme, and could considerably reduce the chance of raising the initial capital at all.

These delays have already meant the loss of a whole agricultural year — a year in which, ironically, climatic conditions were for once ideal. By publicly donating his Nobel winnings to the projected fund, Mr Walesa has, in effect, challenged the government to clarify its attitude to the scheme, and, indeed, to the whole future of Polish agriculture.

Vera Rich

Chinese irrigation

Yangtze to cross Yellow River

CHINA is to accelerate preparatory work on its ambitious project for diverting water from the Yangtze to the Huang-Huai-Hai plains of the north. Construction of the first stage of the project — from the Lower Yangtze to Lake Dongping on the southern banks of the Yellow River — has already begun. The new studies will be concerned with the optimum amount of water that can be transferred, the environmental consequences of the scheme for the lower Yangtze, the overall economic and social consequences of the project and various individual technologies, including the development of large pumps.

The chief objective of the transfer project is to remedy the basic mismatch of China's land and water resources. In the south, the Yangtze, with an annual volume of 980,000 million m³, flows through an area of poor and unproductive soil, while in the north, potentially high quality land cannot be cultivated due to lack of water. The endemic water shortage of the north

under gravity to Tianjin.

Considerable pumping capacity will be needed to effect the 40-m rise from the Jiangdu intake to the highest point (at the Yellow River). Ten major pumping stations are planned with a total installed capacity of nearly 1 million kW. Initial estimates suggest an annual delivery rate of 14,000 million m³ in an average year, rising to 30,000 million m³ in drought years. This will provide new or improved irrigation to some 200 million hectares of land and an additional 2,700 million m³ of water annually for industrial, mining and municipal purposes.

The cost of the diversion has been estimated at a minimum of 10,000 million

yuan (£3,400 million), which, theoretically, will ultimately be recovered in increased agricultural and industrial productivity.

Some concern has, however, been expressed by the United Nations University team that the increase in soil productivity may be less than expected if the ground-water level of the newly irrigated areas is not carefully controlled to avoid an increase of soil salinity.

Another possible hazard is schistosomiasis. The incidence of this disease, which 30 years ago affected an estimated 7 million Chinese, has been considerably reduced by intensive drives to exterminate the snail vectors of the disease, but it remains endemic in southern China. Unless effective countermeasures are taken, the massive transfer of water could also result in the migration northwards of disease-carrying snails.

Vera Rich

French research

Papon looks ahead

THE French Centre National de la Recherche Scientifique (CNRS) is turning the usual means of research support upside down. While continuing to invite researchers to ask for grants, it has set up a series of "crystal ball" groups to identify emerging fields of research, some of which would be selected for special support.

Thus Professor Jean Audouze, director of the CNRS astrophysics institute in Paris, will report soon as leader of a group investigating the future of particle physics in conjunction with astrophysics. Another group is at work on the relationships between chemistry, biology and informatics.

Other such groups are to be set up shortly, as the result of a hot-house seminar of the CNRS directorate held this summer at the Chateau d'Amboise on the Loire. Pierre Papon, director-general of CNRS, and his colleagues concluded that there should be more such future-gazing, and that CNRS should try to concentrate its resources better.

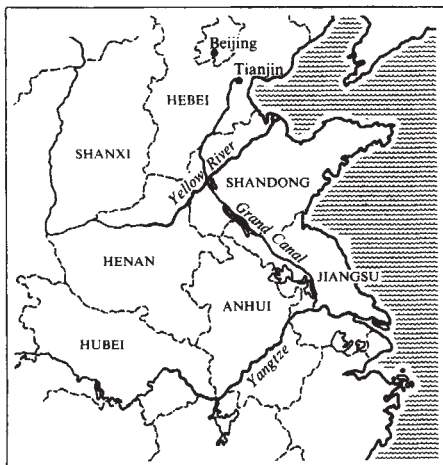
Not that CNRS is disappointed in the past. Speaking at a press conference in Paris on Tuesday, Papon claimed successes for CNRS in 1983 in its contributions to the detection of the intermediate vector bosons, to the development of the free electron laser, in oncogenes (the concept that more than one such gene is necessary to cause cancer) and research claimed to suggest that one of the solar oscillations is driven by gravitational waves from the gamma-ray source Geminga.

In 1984, a laboratory for aquaculture is to be set up at l'Houmeau on the Atlantic coast, in conjunction with the French ocean development organization CNEXO; a materials science institute will go up at Nantes, on the Loire; groups working on drugs-related research will be brought together to form an institute in Paris; and, in the social sciences, an institute on

contemporary society will be established in north Paris, and an interdisciplinary programme set up on "technology, employment, work and way of life".

CNRS has signed conventions on common research objectives with many French institutions, and will sign with more, including industry and regional authorities, said Papon (who is taken with "opening up" CNRS to the outside world); it will offer more grants (known as BDI) to attract high fliers from the *grandes écoles* to train through research, encourage fellowships for European travel and rationalize the system of evaluating the work of CNRS researchers to reduce the amount of paperwork a researcher has to do to maintain his or her funding. Papon will do this on a total budget next year of FF 7,735 million (£645 million), 10 per cent up on 1983.

Robert Walgate



has been exacerbated in recent years by rapid industrial development, particularly in the coal-mining areas of Shanxi province, which has, moreover, led to the pollution of many of the remaining water reserves.

Feasibility studies carried out by the Academy of Sciences and the Tokyo-based United Nations University have focused on the transfer of water from the middle reaches ("middle route") and lower reaches ("east route") of the Yangtze. Although the middle route has a number of technical advantages — in particular, no pumping would have been needed — the State Council decided last March in favour of the east route which, with a length of 1,150 km, is also the shortest. The route will start from a pumping station at Jiangdu on the lower Yangtze and will follow part of the ancient Grand Canal (which will be enlarged and modernized) through lakes Hongze, Luoma, Nansi and Dongpi (serving as flow-control reservoirs) to pass under the Yellow River through a 0.6-km tunnel. It will then flow

Genetics Inst. deal

ALLIED Corporation, the major US chemical manufacturer, has reached an important financial and research agreement with Genetics Institute Inc., the Cambridge (Mass.) company set up three years ago by Dr Mark Ptashne and Dr T. Maniatis, both of Harvard University. A spokesman for Allied said this week that the company has paid \$10 million for a share of the equity in Genetics Institute, and that it would also pay between \$5 million and \$15 million over the three-year initial duration of a research agreement covering new diagnostic products.

The agreement does not at this stage cover other products that may be developed at the Cambridge laboratories, soon to be occupied. Allied would be responsible for the clinical testing and regulatory approval of materials resulting from the joint programme.

British industry

Manufacturing in need of help

THE British Government should spend up to £20 million more each year on encouraging industry to make full use of advanced manufacturing techniques, according to an outspoken new report from the Advisory Council for Applied Research and Development (ACARD). The changes called for range from the establishment of an Advanced Manufacturing Development Service to the suggestion that the Department of Trade and Industry might distribute free robots. The first priority should be to ensure that existing techniques are taken up by industry, says ACARD.

The report, *New Opportunities in Manufacturing*, observes that, even in large companies, design and development frequently accounts for less than 2 per cent of turnover — half the comparable figure for many overseas competitors. Moreover, most of this is spent on product development, rather than improvements to manufacturing techniques.

ACARD believes that industry has failed to take sufficient account of recent advances in manufacturing systems, which have now reached a stage where large-scale integration of automated systems is possible. Resistance to change generally comes from middle management, and at higher levels there is often a failure to recognize advanced techniques as an important long-term investment. ACARD hopes to see suitably qualified board members taking explicit responsibility for manufacturing strategy, and wants these ideas promoted to industry through the National Economic Development Office.

ACARD confirms what many engineers have been saying for some time: traditional engineering degree courses offer inadequate preparation for modern industry. Resources should be channelled towards more training opportunities for technicians, and engineering degree and postgraduate courses should include more about manufacturing techniques. These should be associated with a few strong "centres of excellence" in manufacturing technology that ACARD wants to see established and coordinated by the Science and Engineering Research Council.

On finance, ACARD asserts that there is no shortage of money for investment in new manufacturing technologies, although the financial institutions could do more to ensure that their staff are well informed about production techniques. But the report says that the Department of Trade and Industry's multiplicity of support schemes may actually be confusing some companies, and that some schemes are not well enough supported to make a significant improvement. If programmes were rationalized, more companies might take advantage of them. A principal recommendation is that the department should establish a comprehensive Advanced Manufacturing

Development Service to advise and assist companies. Government could also help by ensuring that public procurement policies encourage companies to use advanced techniques, an idea that has often been put forward.

Many of ACARD's previous reports

have been a significant focus for debate, for example that on links between industry and the universities. As the council reports directly to the Prime Minister, who takes an active interest in such affairs, this report is unlikely to be left to gather dust. But at a time when the government is imposing strict cash limits in other areas of public expenditure, some of ACARD's recommendations may be thought out of tune with the political music.

Tim Beardsley

Gene diagnosis

Yale bungles patent claim

Washington

YALE University may have seriously underestimated the significance of the patented work that led to the production by Enzo-Biochem, under an exclusive licence, of the "Enzo Bioprobe", a non-isotopic method for detecting gene sequences.

David Ward, the principal investigator at Yale who carried out the work, approached the Yale administration in mid-1980 seeking to have his work patented. Yale was then using a non-profit New York firm, the Research Corporation, to handle all its patent matters; the corporation paid the filing fee for patents and sought out licensees in exchange for a significant share of the royalties. The corporation did not consider Ward's work worth the expense of patenting. Ward appealed to several Yale officials, but was turned down.

Ward then began to send out feelers to diagnostic companies. Enzo heard of this and made Yale an offer: Enzo would pay the costs of filing for a patent in exchange for an exclusive licence. Yale agreed.

"After that fact", Ward said, "virtually every major diagnostic company came and

asked for a license." It was, of course, too late, and Enzo has declined to sublicense.

Yale officials defend their action at the time by pointing to the uncertainty that any company would be interested in Ward's work. Robert Bickerton, who now handles patent matters for the university, said that with the sensitivity of the technique at the time — roughly 1,000 times poorer than the standard isotopic methods — the doubt was understandable. The sensitivity has since then improved to the point where it is comparable with the isotopic methods.

In July this year, Yale established its own office to handle patents and has appointed a university committee to review potentially patentable research. It no longer employs the Research Corporation.

Ward does not blame the university for its handling of the affair. "I came along at a time when the university was in a state of flux in how to handle it", he said. And given the university's experience with other patents, he said, their action was understandable. "The return from those was minimal. Why should they assume this was going to be any different?"

Stephen Budiansky

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 30 Sept.	Change
23 1/4	13	Biogen (Switzerland)	14 1/2	14 1/4	-1/4
6 1/4	2 1/2	Bio-Logicals (Canada)	3 1/4	2 1/2	-3/4
16 1/4	7 1/4	Bio-Response (USA)	14	14 7/8	+7/8
19	11 1/4	Cetus (USA)	13 3/4	12 7/8	-7/8
15 1/2	8 7/8	Collaborative Research (USA)	10 1/2	11 1/8	+7/8
39 7/8	15	Damon (USA)	22 1/2	25 1/2	+3
34 1/4	16 1/4	Enzo-Biochem (USA)	25	25 1/2	+1/2
18 7/8	8 7/8	Flow General (USA)	11 1/8	8 3/4	-2 7/8
49 3/4	26 1/4	Genentech (USA)	43 1/2	35 1/2	-8
17 3/4	8 3/4	Genetic Systems (USA)	12 7/8	11 1/2	-1 1/8
23 1/4	12 7/8	Genex (USA)	19 1/2	18 3/4	-3/4
31	19 3/4	Hybritech (USA)	22 3/4	19 3/4	-3
22 1/4	13 3/4	Molecular Genetics (USA)	15 3/4	17	+1 1/4
23 1/4	13 1/2	Monoclonal Antibodies (USA)	15 1/2	14	-1 1/2
73 1/2	42	Novo Industri A/S (Denmark)	61	67	+6
30 1/4	13 1/2	Pharmacia (Sweden)*	29	26 1/2	-2 1/2

Closing prices are for the last Friday of the month. For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. *Nature's* weighted index of biotechnology stocks stood at 221 on 30 September, compared with 232 a month earlier. Data from E.F. Hutton, Inc.

*Pharmacia underwent a 2:1 stock split recently; prices have been adjusted to reflect this.

Closed doors

SIR — Lord Ashby in his review of *Victims of Science* by Richard Ryder (*Nature* 8 September, p.163) refers to "lurid colour plates" depicting animals undergoing experimentation and drawn "for some unexplained reason" from countries other than Britain.

As the secretary of the society publishing the book, and as the photographer concerned, I feel impelled to point out that this is due entirely to the fact that I have never been allowed to witness and photograph any experiment on a live animal performed in a British laboratory, despite numerous requests to do so.

Those who forbid the taking of such photographs on their own premises are hardly in a position to criticize the use of material from other more forthcoming sources, and I challenge British laboratories to allow me to photograph exactly what is taking place in this country.

BRIAN GUNN

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Warsaw release

SIR — On 30 July 1983 I found myself outside the gates of the Mokstow Prison in Warsaw, in consequence of the amnesty decreed by the Polish Parliament. This was two months before the end of the sixteen months' imprisonment to which I was sentenced for sending abroad some letters of critical comment on political developments in Poland. I am now enjoying my freedom, with a greater understanding than before of the aspects of normal life that have real importance. I have also now heard in detail about the many actions taken on my behalf in various countries, of which I was only partially aware in prison.

By this letter I express my sincere gratitude to all those individual scientists, university departments, academics and learned societies who protested that dissent should not be treated as a criminal offence and who sought my early release. Perhaps I may repeat here what I stated during my numerous trials — namely, that I broke neither the existing laws of the country nor the unwritten laws of good citizens. For me personally the actions taken on my behalf showed that I had not been left alone, and they helped greatly to maintain my spirits and to attempt to continue scientific work despite the conditions.

I also see in these protests a valuable demonstration of the solidarity of the international scientific community. I am by no means the only one to have suffered loss of freedom or imprisonment as a result of a political order, and I hope that similar efforts can be made on behalf of all those in such a position. Such actions do not often achieve the desired result immediately, but they nevertheless form links which strengthen each of the scientists involved

and the scientific community as a whole. By contrast, the formal sanctions adopted by some organizations have had little effect on the governments against which they are directed and they do nothing for individual scientists. The vested interest in freedom of speech and enquiry which all scientists have and their personal willingness to defend it seem to me to constitute one of the important forces for peace and progress in the present disturbed world.

RICHARD HERCZYNSKI

Warsaw, Poland

Halley's comet

SIR — In quoting a plethora of variations in the spelling of Halley, David Hughes (*Nature* 11 August, p. 119) has introduced a complicating factor which does not help much in trying to sort out how Halley's name was pronounced in his own day. Old chroniclers certainly did vary in the spelling they used, but so they would, since pronunciation varies depending on local dialect. The variations Hughes mentions come from a variety of sources spread widely over England; the one thing they demonstrate is that spelling names was done phonetically in the days before standardization.

The official documents he quotes make it pretty clear that the pronunciation "Hawley" (to rhyme with "poorly") was customary. Indeed, this is supported by Robert Hooke who, in his manuscript diary, uses the spelling 'Hally' (pronounced Hall + y) rather than "Halley" (which would sound like David Hughes' recommendation of "Hal-ley"). And Hooke was noted for his use of phonetic spelling.

I feel that the argument is not how Halley's name was pronounced in his own day — it was, surely, "Hawley" — but whether we should continue with that pronunciation today. After all, spoken English has changed over the years and must now be different, in some respects at least, from what it was in the seventeenth and eighteenth centuries. Nevertheless my own preference is to use the pronunciation used by Halley's contemporaries, and presumably by Halley himself. After all, this is what we do with his first name Edmond, spelling it with an "o" as he did, instead of using the customary "u".

I do not find the late Sir Harold Spencer Jones' amusing piece of doggeral persuasive. Indeed, in the light of the expectation of cometographers like David Hughes that Halley's comet will make a pretty poor showing at its next apparition, I would support John Mason's new version:

Of all the comets in the sky
There's none like comet Halley,
We see it with the naked eye,
But this time rather poorly.

13 Acorn Avenue, COLIN A. RONAN
Cambridge CB3 8DT, UK

DAVID HUGHES ADDS — The verse quoted in my article should be attributed to H. H. Turner (see *Observatory* 33, 150; 1910).

The Coolidge effect

SIR — In reading the article by J.S. Jones and L. Partridge (*Nature* 18 August, p.484), I thought at first that I was learning something new about the former US president, Calvin Coolidge. However, the explanation of the Coolidge effect was inconsistent with the personality of Mr Coolidge. If the phenomenon described had been called the Harding effect, I would not have given it a second thought!

More importantly, the authors have taken something out of context. An examination of the article in which the Coolidge effect is explained shows that the effect had nothing to do with President Coolidge's sexual habits. The term had its origin in a fable (of which there were many) about Coolidge (D.A. Dewsbury, *Psychol. Bull.* 89, 464):

One day President and Mrs Coolidge were visiting a government farm. Soon after their arrival they were taken off on separate tours. When Mrs Coolidge passed the chicken pens she paused to ask the man in charge if the rooster copulates more than once each day. "Dozens of times" was the reply. "Please tell that to the President," Mrs Coolidge requested. When the president passed the pens and was told about the rooster, he asked "Same hen every time?" "Oh no, Mr. President, a different one each time." The President nodded slowly, then said "Tell that to Mrs Coolidge."

MORTON MALIN

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Catch flies alive

SIR — E.G. Gray gave instructions how to swat flies (*Nature* 25 August, p.678). Here is a method that I have often used to catch spiders, flies, mosquitoes and so on. Cut a piece of thin, stiff, smooth cardboard so that it is large enough amply to cover a plastic beaker. The beakers I use are the white plastic, "throwaway" beakers as used in coffee machines. Hold the beaker near the bottom with your hand well hidden from your "game". Approach the target from the back and behind and quietly place the beaker over the animal, being careful not to squash beaker or beast. Carefully slide the cardboard between the beaker and the wall or glass pane that the animal is sitting on. Keeping the beaker closed remove the combination and dispose at your discretion.

I presume that this works because the beaker is too light and too little structured with too little contrast to be seen or perhaps to be recognized as a threat. Note that shining a light on such creatures will often not trigger any flight. In view of the above perhaps the whiteness of Gray's tissue paper might be part of the trick.

PERCY N. KRUYTHOFF

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Creation versus evolution: no middle way

George M. Marsden*

What is it about society in the United States that led to such complete polarization between the creationists and evolutionists?

In the United States biological evolution is widely regarded as an opposite of divine creation and hence incompatible with traditional Christian belief. This simplified view is so widespread that in 1981 so-called 'creationists' persuaded two states to adopt laws purporting to ensure 'balanced treatment' in public schools by countering any teaching of 'evolution science' with equal teaching of 'creation science'. The very appropriation of the name 'creationist' for the creation science anti-evolutionary movement reflects an insistence, often unquestioned by the public and the press, that there are only two choices in the issue. In fact, however, the creation scientists do not advocate creationism in the general sense of any belief in a divine creator or even in the more limited sense of belief in creation by the God of scripture; rather they defend only one view of creation, that the Earth was created in six 24-hour days and is only some thousands of years old. This view, based on the most literalistic reading of the scriptures, excludes any sort of biological evolution. Self-styled creationists speak of only two views: creation and evolution.

But why should so many Americans, such as state legislators, accept this simplified dichotomy as though there were no mediating alternatives? Even among American evangelical Protestants, such mediating views, usually designated 'theistic evolution' or 'progressive creation', have long been represented. Immediately upon the announcement of Darwin's theory some conservative bible-believers had a ready answer to the suggestion that evolutionary doctrine must undermine faith in a creator: God controls all natural processes through his providential care. The questions raised by biological evolution are therefore not in principle different from those suggested by other natural phenomena, such as photosynthesis. A fully naturalistic account of the process does not preclude belief that God planned or controlled it. So God may have used evolutionary processes as his means of creating at least some of the Earth's species. Moreover, most modern evangelical theologians have agreed that a strict reading of Genesis does not rule out all evolutionary developments. The language of the first chapter of Genesis

might allow for long aeons (days) of God's creative work or it may not have been intended to convey exact scientific information at all.

Even the progenitors of America's fundamentalists tolerated some such latitude. In *The Fundamentals* (1910–15), the publications that signalled the rise of organized fundamentalism, George Frederick Wright contributed one of the essays on evolution. Wright had been a close associate of Asa Gray in defending a theistic version of Darwinism to evangelical audiences. While firmly rejecting evolutionism as a generalized atheistic outlook, Wright insisted that biological evolution could be consistent with God's design so to evolve. Equally striking are the statements made about the same time by Benjamin B. Warfield of Princeton Theological Seminary. Warfield was an inventor of the term 'inerrancy' and a leading proponent of that key fundamentalist doctrine that scripture did not err in any of its assertions. Despite such conservatism, Warfield stressed that evolution and creation were not opposites. For the theist, he observed, evolution was "only a theory of the method of divine providence".

Why then, has opposition to any sort of biological evolution become a test of the faith for so many? The mediating positions have, of course, survived and are even dominant among evangelical academics who are heirs to the fundamentalist movement. Nonetheless, in the current popular American discussions, these positions, as well as their counterparts among Catholics, more liberal Protestants, and Jews, are widely ignored or unknown. Certainly the popular press has done little to dispel the impression of a life or death struggle for survival of two wholly irreconcilable views. So the historical issue I propose to explore is twofold. Why have creation scientists insisted on this polarization, and why have such dichotomized views been so popular in the United States?

"The Bible tells me so"

The Bible is the authority and 'textbook' for the conclusions of creation science. Henry Morris, founder of the most prominent of the current creation science organizations, asserts that "If man wishes to know anything at all about creation . . .

his sole source of true information is that of divine revelation"¹². In recent court cases this theme has been obscured to avert constitutional difficulties. Nonetheless, Morris and his followers agree that it is simply obvious that the first chapter of Genesis refers to creation taking place in 24-hour days and so absolutely precludes evolution. Why do such principles of biblical interpretation persist with such strength? To answer this we must first consider the convergence of two powerful traditions of biblical interpretation.

Millenarianism

The modern premillennial views that have flourished in the United States since the nineteenth century have been based on exact interpretations of the numbers in biblical prophecies. The Bible, such millenarians assume, is susceptible to exact scientific analysis, on the basis of which at least some aspects of the future can be predicted exactly. Seventh-Day Adventists, Jehovah's Witnesses, and the influential dispensational premillennialists among fundamentalists, all treat the prophetic numbers in this way. For such groups it is important to have a biblical hermeneutic that will yield exact conclusions. Moreover, the hermeneutical principles that apply to prophecy should be consistent with those applied to scriptural reports of past events. Dispensationalists have often used the formula 'literal where possible' to describe this hermeneutic. While they do not wish to apply literal interpretations to statements obviously poetical or figurative ("the mountains shall clap their hands"), they do think that, unless we are compelled otherwise, we should interpret the scriptures as referring to literal historical events that are being described exactly. It is not surprising, therefore, that such groups who derive some of their key doctrines from exact interpretations of prophecy should be most adamant in interpreting the first chapter of Genesis as describing an exact order of creation in six 24-hour days.

The influence of these prophetic views goes beyond the bounds of their immediate fundamentalist constituents, as is suggested by the fact that the dispensationalist prophetic volume by Hal Lindsey, *The Late Great Planet Earth*³, was the best-selling book in America during the 1970s.

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The principal creation science organization, the Institute for Creation Research in San Diego, has close ties with this prophetic movement. Moreover, George McCready Price (1870–1963), the main precursor of Morris' young-Earth flood-geology approach, was a Seventh-Day Adventist. Price's whole career was dedicated to confirming the prophecies of Ellen G. White, who claimed divine inspiration for the view that the worldwide flood accounts for the evidence on which geologists build their theories.

Protestant scholasticism

Not all creation scientists are millenarians, however. Another formidable tradition in American Protestantism that has often supported strict views on Genesis One and has influenced both American fundamentalism and popular American conceptions of scripture is Protestant scholasticism. This tradition has been articulated most prominently by the Princeton theologians, such as Benjamin Warfield. The substance of this view of the inerrancy of the scriptures — that because the Bible is God's word it must be accurate in matters of science and history as well as in doctrine — was already incorporated into much of the scholastic Protestantism of the seventeenth century and was common in many quarters of nineteenth century American Protestantism. Belief in the inerrancy of the scriptures did not entail that they should always be interpreted as literally as possible, a fact which is demonstrated by the allowance for long 'days' of creation by some Princetonians. Nonetheless, the emphasis on the scientific accuracy of scriptural statements was conducive to views of those who insisted that the first chapter of Genesis referred to literal 24-hour days.

A good example is the Lutheran church — the Missouri Synod. For reasons no doubt related both to their Protestant scholastic tradition and an immigrant group's determination to resist infection by modern American theologies, the leading theologians of the Missouri Synod insisted on a most conservative view of the scriptures throughout the first half of the twentieth century. The Holy Spirit dictated or suggested to the writers the very words of the scriptures, therefore these words of God have divine properties. Since God would speak with great accuracy, it seemed to the Missouri Synod interpreters that the days described in the first book of Genesis must be 24 hours long. So evolution was "atheistic and immoral" and theistic evolution was inconsistent with both the scriptures and true evolution. When in 1963 Henry Morris first organized the creation science movement, he found enough allies from the Missouri Synod to make up a third of the original steering committee.

Rational scientific Christianity

A desire to establish a firm rational and scientific basis for Christian belief has been common to the prophetic millennial and

the scholastic traditions and has related them to each other. In the eighteenth and nineteenth centuries particularly, defenders of Christianity assiduously collected evidence from the natural sciences to confirm "truths" revealed in the scriptures. Nineteenth century American apologists, whether scholastic or millenarian, typically based their arguments on explicitly "Baconian" principles: cautious examination of evidence that everyone could observe through common-sense procedures.

Crucial to the creation science movement is the desire to restore this harmony of science and scripture which the twentieth century intellectual climate had seemingly shattered. Henry Morris made this point explicitly in his first book, *That You Might Believe*¹. While acknowledging that Christian truths must ultimately be based on faith and that he would accept the Bible "even against reason if need be," Morris emphasized that the Bible "in no way does violence to common sense and intelligence." Many twentieth century people regarded Christianity "as outmoded beliefs, conceived in superstition and nurtured by scientific and philosophical illiteracy". Morris, by contrast, was sure that biblical beliefs would satisfy even his habit, cultivated by his engineering background, of "requiring satisfactory evidence and proof of all that they accept as fact".

Buoyed by this confidence in the Bible, Morris proceeded to illustrate "the great number of scientific truths that have lain hidden within its pages for 30 centuries or more, only to be discovered by man's enterprise within the last few centuries or even years". These 'facts' included statements that the stars "cannot be numbered," or that the psalms directly described evaporation, wind, and electrical discharges as the cause of rain (Psalm 135 v. 7). The creation science movement grew out of this impulse. While not claiming actually to prove that Christianity must be true, it seeks decisive evidence confirming biblical statements. So, not only do creation scientists assemble scientific evidences pointing to a worldwide flood, they sponsor expeditions searching for Noah's ark.

The whole enterprise relates to a distinctive view of the scriptures themselves. Fundamentalists and their allies regard the Bible as filled with scientific statements of the same precision as might be found in a twentieth century scientific journal. God, they assume, would not reveal himself any less accurately. The Bible, in the fundamentalist and scholastic traditions, is regarded as a book of fixed 'facts'. This view of the scriptures as a series of scientifically accurate propositions has invited the literalist interpretation that allows biblical language as few ambiguities as possible. For instance, a common argument against the evolution of species is that Genesis asserts that plants and animals should produce "after their kind". This phrase is usually regarded as precluding one species ever

producing another. Similarly, a well-known dispensationalist argues that the statement in Genesis ch. 2 v. 7, that man was created "out of the dust of the ground" could not allow for evolution from the primordial dust "since it is to dust that man returns — and this is not a return to an animal state (Genesis ch. 3 v. 19)"⁵.

Common sense

Scholars from other traditions might find such thinking incredible, applying linguistic standards of one age to another. Nonetheless, there can be no doubt that in our age such thinking is widely regarded as common sense. Fundamentalists and kindred religious movements have made strong claims to stand for common sense.

The Bible, according to the democratic popularization of this view, is best interpreted by the naive readings that common people give it today. In modern America, common sense is infused with popular conceptions of straightforward empirical representations of what is really 'out there'. Mystical, metaphorical, and symbolical perceptions of reality have largely disappeared. Instead, most Americans share what sociologist Michael Cavanaugh calls an "empiricist folk epistemology"⁶. Things are thought best described exactly as they appear, accurately with no hidden meanings. Such folk epistemology is close to that which works best for engineers, straightforward, consistent, factual, with no nonsense. In fact, there are an unusual number of engineers in the creation science movement.

Most contemporary scientists have difficulty understanding the appeal of alleged scientific arguments of creation science to popular common sense. Evolution may have scientific experts on its side, but it strains popular common sense. It is simply difficult to believe that the amazing order of life on Earth arose spontaneously out of the original disorder of the Universe. The development of specific mechanisms such as the eye through blind chance also stretches common credulity. Could everything appear to be so ordered just by accident? The length of time it would take for the present order of life to arise from disorder is staggering and stretches popular conceptions of probability. As a common sense argument, an anti-supernaturalistic evolutionary outlook is far less compelling than the traditional explanation.

As to the fact that so many experts agree on the truth of evolution, experts have often been wrong. Besides, the experts contradict each other, so that the lay person has no obligation to believe them. Moreover creation scientists can produce their own experts, as their organizations emphasize. In addition, however, people should be "deciding for themselves in a reasonable way". Audiences in church basements are told to go "see for themselves" fossil evidence that supposedly undermines evolution. "Let's decide upon a method by which we can resolve the

controversy," says a typical appeal, "set up definitions, then examine the evidence"¹⁷.

The American folk epistemology, then, is by no means anti-scientific in principle. Rather it is based on a naive realism plus popular mythology concerning proper scientific procedure and verification. These procedures are essentially Baconian, favouring simple empirical evidence. The view of science is optimistic and progressive, the real science will eventually reach the truth, although it may be led off the track by prejudice.

Post-Civil War cultural crisis

The popular appeal of uncompromising anti-evolutionists results not only from the coincidence of their hermeneutical and apologetic assumptions with much of American folk epistemology but also from their ability to convince their followers that anti-evolution is crucial to the future of civilization. Militant anti-evolutionists are almost all Northern European Protestants. Many of them have emphasized vigorously America's Christian (Protestant) heritage. A sense of cultural crisis, typically described as a turning from Christian to secular civilization, seems an important factor in raising the stakes of the anti-evolution effort and hence reducing the likelihood of compromise.

This combination of beliefs seems more characteristic of the United States than of other countries, and more characteristic of the south than of the rest of the nation. The irreconcilability of evolution and the Bible is a widely-held popular belief in the south that dates from before the creation science movement.

The easy answer to explaining the strength of anti-evolutionism in the south is the prevalence of 'old-time religion', the popular resentment of experts, and the relatively low levels of education of many southern Bible-believers. These factors are certainly important, although they do not explain why a belief in the dangers of evolution gained an elevated status in southern folk-religion. An interesting question is why anti-evolution became a standard test of the faith among southern evangelicals earlier than it did among northern fundamentalists. Already by the 1880s several southern theologians had lost their professorships because of their most cautious efforts to reconcile the scriptures to biological evolution. Much of the popular religious press made the issue crystal clear, asking "are we creatures of God or offspring of the apes?"

Why did southern religious groups thus try to bolt the door on even the most modest accommodation between creation and evolution? The answer is that a number of factors converged. First are the dynamics of the southern white church and religious life after the Civil War. The war brought the restoration of the Union but not the reunion of the churches. Southern Christians had to justify this continued

separation from their former brethren. The most likely principal explanation was that their northern counterparts had been infected by a liberal spirit, shown originally by their unbiblical attacks upon slavery. Southerners were thus alert for any other trends towards laxity in Yankee religion. The continued separation was justified by the mounting conviction of southerners that they were the only remaining representatives of true religion.

Such justifications of separation from the northern churches were an integral part of the southern glorification of the lost cause. Although southerners had lost the war on the battlefield, they were determined to win the war of ideas. The effect of this determination was to preclude change in any area and to celebrate whatever had been dominant before the war. This southern determination arose almost simultaneously with the rapid spread of evolutionary ideas in the north. So the widespread belief in the value of change became particularly anathema in the southern thought. Evolution, or change of any sort, could be only a decline.

Such circumstances may have been sufficient to ensure some popular opposition to any evolutionary doctrine. In addition the theologians' stance on the issue of Genesis and biological evolution was reinforced by a firm commitment to a scholastic literalist hermeneutic. Southern theologians, like most early nineteenth-century American churchmen, viewed the Bible as a collection of factual statements. Moreover, they were particularly inclined to a literalistic hermeneutic because of the slavery controversy. The Yankee reading of the Bible as condemning slavery seemed to southerners to involve abandoning the letter of the text for the alleged spirit. Committed to the letter of the scriptures regarding slavery, such southerners were hardly in a position to play fast and loose with other passages that might be reinterpreted in the light of modern progress.

Fundamentalism after 1918

The fundamentalist campaigns against evolution in the 1920s bought the supposed dichotomy to the national level. Before the First World War, anti-evolution does not appear often to have been a test of the faith outside the south, except among sectarians. Probably most conservative Protestants had the impression that evolution and the Bible were irreconcilable opposites, but a large enough number of their leaders saw the problems inherent in this stance to prevent it from becoming a test of fellowship.

As we have seen, even *The Fundamentals* of 1910 to 1915 did not absolutely preclude all evolutionary views. During the 1920s, however, anti-evolution became increasingly important to fundamentalists and eventually became an essential hallmark of the true faith.

The rise of the anti-evolution issue in fundamentalism was related to the

convergence of several forces that took their exact shape when precipitated by the catalytic action of the American experience in the First World War. The war brought out sharp conflicts between liberal, or 'modernist', and conservative Protestants. Fundamentalists made the most of the extravagant anti-German propaganda by pointing out that German theology was the source of much modernist thinking. Common to German theology and German *kultur*, they said, were evolutionary philosophies. This "might is right" ideology had led to disaster for that civilization, which had lost all sense of decency. Evolution, moreover, had turned Germans away from faith in the Bible. The same thing that had happened to Luther's Germany could happen to Protestant America. Civilization itself was at stake.

The campaign only needed a leader to become a national sensation. William Jennings Bryan played that role as no one else could have. In estimating the reasons for the rise of an idea one must not underestimate the role of a charismatic personality. The battle for anti-evolution, the Bible, and civilization was a cause whose time had come; but it is doubtful that it would have become so deeply engraved in American thought had it not been for the colourful leadership of Bryan. If nothing else, Bryan's presence ensured wide press coverage, which of course always invited further simplifications of the issue.

Bryan's own understanding of the connection between biological evolution and the dangers of evolutionary philosophies to society was an unusual one. In his view, evolutionary social views led to social Darwinism and hence to anti-progressive politics and the glorification of war. His followers, however, were not especially concerned with the details of the threat posed by evolution to civilization. They were convinced there was a threat to traditional beliefs which resulted from the spread of naturalistic, evolutionary-developmental philosophies. This supposition was not entirely fanciful. Bryan and his cohorts were aware in a general way of the same secularizing trends associated with evolutionary naturalism in philosophy that were being pointed out by many of their intellectual contemporaries.

One strength of the fundamentalists' position was that they could relate this threat to civilization directly to the abandonment of the Bible as a source of authority and truth. This linkage became most clear in the question of biological evolution. Here again, the fundamentalists were pointing to a real phenomenon of major cultural significance. American college students were forsaking traditional faith in the Bible in droves. Science courses, especially those that taught naturalistic evolution, were the leading contributors to this revolution. In fact, nearly two-thirds of the nation's biologists professed not to believe in a personal God or in immortality. The teaching of

evolution was, then, a real contributor to a trend that many considered to have ominous implications for the future of civilization.

The perception of such stakes invited the sort of polarization of the issue that we have been discussing. Bryan's appeal to the quasi-populist rural resentment of experts, especially in the south, added to the oversimplifications. Bryan's own case is especially revealing since the private Bryan and the public Bryan of the 1920s seem to have disagreed on how simple the issue was. Bryan himself held to a somewhat moderate interpretation of Genesis. As Darrow elicited from him at the Scopes trial, Bryan believed that the first chapter of Genesis might allow for an old Earth, a belief that was not unusual among fundamentalist leaders. Bryan even confided just before the trial that he agreed that one need have no objection to "evolution before man"⁸. Yet in his public speeches, Bryan had been allowing no compromise. "The so-called theistic evolutionists refuse to admit that they are atheists," he argued. Theistic evolution, he added, was just "an anaesthetic administered to young Christians to deaden the pain while their religion is being removed by the materialists"⁹. Any public concession to the feasibility of evolution, he explained privately, would give the opponents too much of a presumptive argument for the evolution of humans. Here we see the impact of a skilled popular leader in polarizing an issue. Convinced that the matter was of unparalleled importance, Bryan was not going to allow his constituency to be distracted from the warfare by the fine distinctions of mediating positions.

The warfare metaphor

Exacerbating the tendencies to polarization that arose from the convergence of all the factors mentioned has been the sheer power of military metaphors. For over a century, warfare has been the dominant popular image for considering the relationships between science and religion, evolution and creation. Journalists and historians relish reporting a good fight.

In describing the relationship between Darwinism and religion, argues James R. Moore in *The Post-Darwinian Controversies*¹⁰, the military metaphor was first promoted by the opponents of religion. In fact, ever since the famed encounter between Bishop Wilberforce and T. H. Huxley of 1860, there was something of a warfare between some churchmen and certain anti-supernaturalist evolutionists. Given the many suggestions that creation and evolution might be complementary, however, these conflicts might easily have been resolved or confined. Militant opponents of the whole Christian cultural and intellectual establishment, however, made the most of the conflict. Darwin's personal difficulties in seeing how theism could fit with his theories lent aid to their cause. Ac-

cordingly, Victorian polemicists like T. H. Huxley and historians such as John William Draper and Andrew Dickson White reinforced the idea that the whole history of the relations between science and religion was one of "warfare". As the statistics on the low number of traditional theists among early twentieth-century American biologists show, the weapon of evolutionism was indeed taking a heavy toll in this warfare on Christianity.

Given this actual hostility of many evolutionists towards traditional Christianity, it is not surprising that some Christian groups replied in kind. Particularly this was true of groups that already saw most of reality through warfare imagery. Sects are notorious on this score. Immigrant groups and southerners each had their own reasons to view themselves as conducting at least a cold war with the surrounding culture. Anti-evolution hostilities, however, did not reach nationwide proportions until the rise of fundamentalism in the 1920s. Fundamentalism was a peculiar blend of sectarianism and aspirations to dominate the culture. It was a coalition of conservative and Protestant traditions with militancy as its most conspicuous, unifying feature. As Richard Hofstadter observes, "The Fundamentalist mind . . . is essentially Manichean; it looks upon the world as an arena for conflict between absolute good and absolute evil, and accordingly it scorns compromises (who can compromise with Satan?)"¹¹. William Jennings Bryan's refusal to admit the possibility of limited evolution publicly for fear of giving a weapon to the enemy illustrates this tendency.

The evolutionary explanation

William Allen White said of Bryan that he was never wrong in political diagnosis and never right in prescription. We might say the same of the creation science movement that has been heir to his work. They have correctly identified some important trends in twentieth-century American life and see that these trends have profound cultural implications. They point to the revolution that has brought the wide dominance in American academia and much other public life of anti-supernaturalistic relativism. Evolutionary theory has, as we have seen, often been used to support such an outlook. Carl Sagan's immensely popular television series *Cosmos* furnished a telling example. "The cosmos is all there is, there was, or ever will be," he states in his opening sentence¹².

Such views are, of course, philosophical premises rather than conclusions of scientific inquiry. No conceivable amount of scientific evidence could settle such an issue. Nonetheless, both sides make the same mistake in debating such questions. Both fundamentalistic anti-supernaturalists, such as Sagan, and their creation scientist opponents approach the issue as though it could be settled on the grounds of

some scientific evidence. In each case, the oversimplification of the issue reflects widespread overestimation in American culture of the possible range of scientific inquiry.

Beyond such overestimation of the process of science in general is the peculiar role that "evolution" has come to play in the anti-supernaturalist cultural and intellectual revolution. Both anti-supernaturalists and their creation scientist opponents have reflected common parlance when they have spoken of "evolutionary science" as equivalent to "naturalism" — that is, a view that the Universe is controlled by natural forces insusceptible to influence by any ultimately supernatural plan or guidance.

Moreover, as David N. Livingstone suggests, evolution has become an all-explanatory metaphor in modern culture. It has become a "cosmic myth — an idea which purports to provide, for example, guidelines for ethics and a coherent account of reality". All aspects of being and experience are explained according to evolutionary, developmental, or historical models. Often these are presented as complete accounts of the phenomena involved or as the only meaningful accounts that are available to humans. Evolution is, of course, a model with valuable explanatory powers; but it is worth asking, as Livingstone does, where we have any adequate basis for making this metaphor the foundation for an all-inclusive view of the world¹³.

In any case, creation scientists are correct in perceiving that in modern culture "evolution" often involves far more than biology. The basic ideologies of the civilization, including its entire moral structure, are at issue. Evolution is sometimes the key mythological element in a philosophy that functions as a virtual religion. Given this actual connection with an ideology that opposes traditional Christianity, it is all the more difficult for many conservative Christians to see that the biological theory is not necessarily connected with such a philosophy. Dogmatic proponents of evolutionary anti-supernaturalistic mythologies have been inviting responses in kind. □

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Nobel Prize to Barbara McClintock

This week's Nobel Prize for Medicine has gone to an octogenarian plant geneticist best known for her work during the past half century on transposable genetic elements.

THIS year's award of the Nobel Prize for Medicine to Dr Barbara McClintock, announced last Monday, is in that minor tradition of the Swedish Academy which singles out for distinction those whose significant work is described as "ahead of its time", or whose significance is only appreciated with the passage of time. The late Dr Peyton Rous's prize for his discovery more than half a century earlier of an association of viruses and malignancy is of the same kind.

In McClintock's case, part of the difficulty seems to have been that of accommodating within the framework of genetics developed by T. H. Morgan, his associates and his successors, her observations (in the 1940s) of the sometimes wayward behaviour of inheritable genetic elements. This is now interpreted as the occurrence of movable (or even invertible) genes within a single set of plants, a behaviour recognized not merely in plants (McClintock worked principally with maize) but in *Drosophila* and many other organisms. It is therefore significant that one of her first contributions to genetics (in 1931) was a demonstration that genetic recombination at meiosis, leading to a reassortment of the genes inherited from separate parents, is accompanied by the physical transfer of material from one chromosome to its partner. This early observation, which may be regarded as the foundation of cytogenetics, was at the time a compelling proof that genes are indeed parts of chromosomes.

McClintock's work on transposable genetic elements in maize, based on observations at the University of Missouri during the 1930s, is mainly concentrated into the period beginning in 1944. She convinced herself that some genes in the maize genome were outwardly unstable because of the migration of transposable genetic elements to their loci. In a review of a biography of Barbara McClintock published earlier this year (W. H. Freeman), Professor J. R. Fincham wrote of these observations (see *Nature* 304, 377; 1983):

In the context of the prevailing cytogenetical orthodoxy (an orthodoxy to which McClintock's work had previously lent so much support) these were astonishing results indeed. An example of the induction of high mutability at one locus by a dominant element at another locus had already been reported by Marcus Rhoades, but the possibility of transposition was new and very difficult for most geneticists to accept. To make the problem of communication worse, McClintock added another controversial

dimension to her interpretation. She had become convinced that the mutation-like and genetically controlled changes in gene activity that she was observing were representative of processes that controlled gene switching in normal development.

At the 1951 Cold Spring Harbor Symposium, McClintock outlined the main results and conclusions from her six years of intensive work. She met with almost total incomprehension. Few understood what she was saying and fewer still were prepared to accept it. Some, I am told, simply failed to see how one person could possibly have done all the work necessary to establish the conclusions that she now presented in such concentrated form.

Although unsuccessful at persuading others to follow her interpretations, McClintock was certainly not shunned; she was a member of the National Academy of Sciences at the age of 41 and president of the Genetics Society of America in 1945. One example of how underappreciated was her work is that when in 1962 Jacob and Monod wrote up their operon model of



genetic control in bacterial systems, they did not mention McClintock. After she had drawn their attention to the parallels between her two classes of genetic controlling elements (a term she coined) and bacterial regulators and operators, they immediately acknowledged her contributions.

Only in the 1960s did it become clear that McClintock's proposals of movable genetic elements were more than a fancy explanation for the extraordinary variegations of colour in maize kernels, her principal laboratory tool. First came evidence from bacterial and phage genetics that strongly suggested the insertion of genetic material at new locations. By the early 1970s, insertion sequences and transposons had been discovered (as well as the associa-

tion between them and the spread of antibiotic resistance).

More recently, the existence of movable genetic elements in higher organisms has become certain, and groups at the Cold Spring Harbor Laboratory and the Carnegie Institution of Washington, among others, have been able to confirm at a molecular level much of what McClintock had intuitively deduced. She seems to have erred only in supposing that genetic rearrangements have an important role in development; with a few important exceptions (the generation of antibodies and the switching of mating type in yeast, for example) that does not seem to be the case.

Now, Barbara McClintock lives a spartan and rather secluded life on the campus of Cold Spring Harbor Laboratory in Long Island, New York, where she has been since 1942. Not that she was ever on the staff of the laboratory. Instead, she was supported by the Carnegie Institution of Washington as a staff member until 1967, when she reached the aged of 65, and has been a Distinguished Service Member since then.

Until four years ago she lived in two rooms of what used to be stables. Now she occupies a small apartment attached to one of the dormitory houses of the Cold Spring Harbor Laboratory, but still lives sparsely, in spite of having been awarded a MacArthur Fellowship a few years ago. (She then, however, became a car owner for the first time.) At the age of 82 she continues to use her laboratory most of the time and was still planting out maize last year. Now as always, she has no co-workers, no technicians and no secretary.

Nor does she publish papers. Very little of McClintock's data have ever been published except in the year books of the Carnegie Institution (from 1946) and in Cold Spring Harbor Symposia papers.

With this background, McClintock is concerned that her stocks of genetically categorized maize seeds and her data should not be lost. With the help of the Cold Spring Harbor Laboratory, arrangements have been made to ensure that the techniques of planting maize will be learned by a trained geneticist, and that the breeding stocks will be maintained in good shape.

In deciding whether or not to award the Nobel Prize for Medicine to McClintock alone, those responsible have clearly decided that her contributions have been so singular that the molecular heirs to her ideas should stand aside for a great lady.

Neurophysiology

Trophic factors and postsynaptic activity in synapse formation

from T. Lomo

THE adult neuromuscular system continues to offer new and increasingly detailed information about synapse formation. Those interested in development may even trick the system into recapitulating synaptogenesis. All you need to do is to transplant a nerve on to a nonsynaptic region of another muscle, give it two weeks to grow into the muscle and then make the muscle receptive by cutting its own nerve. This stimulates the transplanted nerve to form new ectopic synapses that begin to transmit impulses within two to three days and reach maturity within two to three weeks. Moreover, you may cut the transplanted nerve at different stages of synaptogenesis and study the fate of the immature postsynaptic site in the presence (or absence) of muscle activity evoked by chronic direct stimulation of the denervated muscle. Such experiments — examples of which have just appeared in *Nature*^{1,2} — have confirmed and extended observations obtained in cultures of nerve and muscle cells and in normal embryonic development.

Denervation elicits the appearance of extrajunctional acetylcholine (ACh) receptors which subsequently cluster quickly underneath foreign nerve terminals as these sprout, probably as a result of the release of ACh-receptor-aggregating peptides^{3,4}. Now, Hume, Role and Fischbach¹ report in this issue of *Nature* that growth cones of cholinergic neurones release ACh even in the absence of target myotubes. Earlier reports⁵ had presented evidence that substances moved by axonal transport and released by nerve stimulation increase ACh sensitivity of organ-cultured skeletal muscles. Axonal sprouting may be attributed to factors released by the muscle as a consequence of the denervation⁶. Within hours of ACh-receptor cluster formation synaptic transmission becomes sufficiently effective to evoke muscle action potentials and contractions despite the fact that the terminals at this stage release few quanta of ACh and postsynaptic folds are absent. Other immature features, however, such as multiple innervation, lack of acetylcholinesterase and the presence of long ionic channel open times (4 ms) potentiate the postsynaptic response.

Up to this stage synapse formation can occur in the absence of impulse activity. Further maturation, however, which includes the appearance of acetylcholinesterase and growth of some junctions and elimination of others, requires activity. This can be demonstrated by transplanting the foreign nerve at an early stage, and then observing that postjunc-

tional maturation continues if the muscle is stimulated directly, but is arrested if it is left inactive⁷.

An important paper appearing in the October 6th issue of *Nature*, from Brenner, Meier and Widmer², extends these observations. They cut the transplanted nerve at an early state when ACh receptor channel open time is long (4 ms) and postjunctional folds and acetylcholinesterase activity are absent, and went on to show that during subsequent direct stimulation of the muscle the denervated junctions develop normally by acquiring short channel open times (1 ms), extensive membrane folding and junctional acetylcholinesterase activity. Unfortunately, they do not describe the fate of these junctions in the absence of stimulation, but since many other junctional properties are arrested in their development in the absence of activity, we may presume that this is also the case for the properties they studied.

From these observations, a clearer picture of synaptogenesis emerges. When nerve terminals first contact a receptive muscle fibre, they induce many changes ('imprints') which require muscle activity to become expressed at a later stage. It seems likely that the early imprints are caused by trophic substances and/or contact interactions with the nerve and that the later changes result from unknown interaction between these imprints and evoked muscle impulse activity. Not only activity as such but also pattern of activity may influence synaptic properties. Motor endplates in fast and slow muscles have different morphological and physiological properties^{8,9}. This may be related to the different impulse patterns normally transmitted across these junctions since 'fast' and 'slow' stimulus patterns induce synthesis of acetylcholinesterase molecular forms at denervated rat soleus endplates that are similar to the forms in fast and slow muscles respectively¹⁰. This is a satisfying outcome of the debate on whether neurotrophic influences or muscle activity control muscle properties. Clearly, with respect to synaptogenesis, trophic factors and muscle activity are both essential. With respect to extrajunctional properties, however, the situation is still unclear.

Of course, many questions remain. For example, a given foreign nerve will readily make ectopic synapses with the soleus, but hardly at all with the extensor digitorum longus¹¹. What surface property is responsible for the peculiarly high receptivity of the soleus muscle? What is the structural correlate of the neurally induced imprint, and how long does it persist after removal

of the nerve? Does it reside in the basal lamina of the synaptic cleft, which has been shown to have an important organizing role during regeneration of pre- and postsynaptic specialization¹²; or in the muscle fibre proper? A regenerating soleus nerve will easily reinnervate the original endplates of ectopically innervated fibres when returning soon (1–2 weeks) but not late (1–2 months) after denervation¹³. Apparently denervated endplates may gradually lose some properties, suggesting that the neural imprint may be long-lived — but not everlasting. Ectopic innervation affects the properties of denervated original endplates lying several millimetres away either by evoked muscle activity and/or by some long-distance action of a trophic influence¹⁴. However, ectopic innervation appears to be much more successful in maintaining junctional ACh-receptor aggregates than direct chronic stimulation even though the stimulation effectively removes extrajunctional receptors¹⁵. Although other interpretations are possible, this may signify that trophic influences can act over some distance. □

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Corrigenda

IN 'Discovery of a new albatross' (*News and Views* **305**, 181; 1983) the reference to the original article was omitted. The discovery was first reported by Roux, J-P, Jouventin, P., Stahl, J-C. & Weimerskirch, H. in *L'Oiseau et la R.F.O.* **53**, 1; 1983.

CORNELIUS Hoenselaers and Martin Walker of the Max-Planck-Institut für Physik und Astrophysik, Garching bei München, have kindly pointed out that in 'Matters of moderate gravity' by Virginia Trimble (*News and Views* **305**, 10; 1983), the fifth sentence in the third paragraph would be more accurate if written "That is, the Kerr-Newman metric is the only stationary axisymmetric single, isolated black hole solution possible within GR." □

Neuropharmacology

Pathophysiology of schizophrenia — causal role for dopamine or noradrenaline?

from L. L. Iversen, G. P. Reynolds and S. H. Snyder

IN a recent article in *Nature*, Hornykiewicz¹ has provided a thorough and thoughtful review of the abundant literature on the role of catecholamines in schizophrenia and advocated a role for noradrenaline, while criticizing evidence for dopamine involvement. In discussions of catecholamines in schizophrenia, all too often two separate issues are muddled together. One is the role of various neurotransmitters in mediating the therapeutic effects of drugs in schizophrenia; the other is the possible alterations in neurotransmitters or their regulatory enzymes in schizophrenic brain. The evidence on the former issue is strong, that on the latter weaker.

Much of the evidence for a role for noradrenaline in schizophrenia reviewed by Hornykiewicz relates to the localization of noradrenaline in limbic 'emotional' parts of the brain and behavioural evidence indicating that noradrenaline is involved in stress responses. Though this fits a role for noradrenaline in schizophrenia, it is also consistent with noradrenaline involvement in affective disorders, where the evidence is in fact stronger: of course, evidence of a similar sort with comparable or stronger documentation applies as well for dopamine. In extensive studies of catecholamines in postmortem samples of human brain the concentrations of dopamine are found to be at least as high as, if not higher than, those of noradrenaline in several of the limbic forebrain areas in which Hornykiewicz indicates that noradrenaline is predominant (for example, amygdala, nucleus accumbens). A sub-dissection of the nucleus accumbens, as defined by Brockhaus², has failed to reveal an area in which noradrenaline is present in excess over dopamine.

As for specific abnormalities of noradrenaline or dopamine or their synthetic or degrading enzymes or receptors in schizophrenic brain, there are published data with regard to both catecholamines. Changes have been found in levels of dopamine and noradrenaline and/or their metabolites in schizophrenic brain or spinal fluid. However, on balance the changes have been modest and not consistently reproduced in different laboratories. Moreover, in the great majority of instances it has not been possible to rule out the strong likelihood that observed changes are due to treatment with neuroleptics. In the case of the very consistently observed increase in number of dopamine receptors in schizophrenic brain^{3,4}, there is evidence that the changes

are related to neuroleptic drug treatment⁵. Furthermore, one of us (G.R.) has found recently that significantly increased levels of noradrenaline (and dopamine) in cerebrospinal fluid from patients with paranoid schizophrenia ($n=28$) were seen only in those patients who were receiving neuroleptic medication (about half) and the noradrenaline levels were independent of psychiatric ratings⁶.

Thus, there are no reproducible changes in postmortem brain indicating that either noradrenaline or dopamine is causally involved in the symptoms of schizophrenia. What is impressively established is that dopamine is involved in the influences of drugs on schizophrenic symptoms. The therapeutic efficacy of neuroleptics is closely correlated with their relative potencies in blocking one subtype of dopamine receptor, the D_2 receptor^{7,8}. Hornykiewicz points out that numerous neuroleptics are potent α -adrenergic receptor blockers. While this is true and while some neuroleptics are also very potent muscarinic anticholinergic, antihistamine and antiserotonin drugs, there is no correlation between their potencies in blocking any of these other receptors and therapeutic effects⁹. Moreover it is unlikely that the low parkinsonism-inducing potency of neuroleptics such as thioridazine is associated with noradrenaline receptor blockade. Thioridazine is only as active an α -adrenergic antagonist as the principal neuroleptics mentioned above, and its unusually potent anticholinergic actions probably account for the low incidence of extrapyramidal side effects¹⁰.

The mediation of neuroleptic therapeutic effects by dopamine receptor blockade is probably as well established as is the mechanism of action for any group of drugs in psychiatry and for that matter in general medicine. Amphetamines, which act by releasing catecholamines, predominantly dopamine, dramatically and selectively worsen schizophrenic symptoms in some patients¹¹. Dopamine is more likely to be involved than noradrenaline, since L-dopa, which is converted in the brain largely into dopamine with little if any noradrenaline formed, also exacerbates schizophrenic symptoms¹². Thus, one can titrate schizophrenic symptoms up or down by modulating synaptic levels of dopamine. This by no means implies a causal role for dopamine in schizophrenia. However, it does suggest that dopamine systems may be fairly closely linked to whatever is fundamentally aberrant in schizophrenic brain. Future effort needs to be directed to understanding the nature of

the relationship between dopamine and other modulatory neuronal systems in brain, a relationship which one would expect to be altered by neuroleptic drugs. □

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Oleh Hornykiewicz replies:

I AM pleased that Iversen *et al.* make such a strong case for the need of discriminating between the role of brain dopamine receptor blockade in the antipsychotic activity of the antidopaminergic neuroleptics — which no one would dispute — and the rather hypothetical role of dopamine in the pathophysiology of schizophrenia. The introductory paragraphs of my commentary were actually aimed at cautioning against this grave mistake. Strangely, despite their well founded warning against the misuse of the 'drug evidence', towards the end of their communication Iversen *et al.* come close to falling victim to this mistake by attaching so much weight to the fact that in schizophrenic patients, "one can titrate schizophrenic symptoms up or down by modulating synaptic levels of dopamine" with drugs such as amphetamine or L-dopa. Although Iversen *et al.* are swift to add that this "by no means implies a causal role for dopamine in schizophrenia", to contemplate such drug effects in isolation from other evidence is not very helpful to future research. As I have already explained one also can titrate Parkinsonian (and many other) symptoms up or down by modulating the levels of brain acetylcholine with cholinergic drugs, but it would not have resulted in any

breakthrough if we had been content with the (as such very correct) statement that the cholinergic brain systems "may be fairly closely linked to whatever is fundamentally aberrant" in the Parkinsonian brain: it is precisely this "whatever is fundamentally aberrant" that opened up completely new possibilities.

Turning to the comments directly related to the theme of my article, that is noradrenaline and schizophrenia, Iversen *et al.* note that "changes have been found for levels of dopamine and noradrenaline and/or their metabolites in schizophrenic brain of spinal fluid", but that "on balance the changes have been modest and not consistently reproduced in different laboratories". This statement certainly is true for the various studies reporting changes, or lack of changes, in dopamine and/or its metabolite homovanillic acid¹⁻¹⁰, or dopamine receptor sites^{9,11-16}, from which no consistent picture can be derived. This is not so, however, with the reported changes in noradrenaline levels in schizophrenia. First, there is a consistent body of published cerebrospinal fluid results, all showing the same: a significantly above-normal level of cerebrospinal fluid noradrenaline in schizophrenic patients, especially those belonging to the paranoid subgroup¹⁷⁻²⁰. Second, it is this subgroup, the paranoid variety, in which an impressive increase in brain noradrenaline has been reported²¹⁻²³; other schizophrenic subgroups, if analysed, did

not show this change^{7,22,23}. Thus, the question of noradrenaline in schizophrenic brain crucially depends on this diagnostic distinction²⁴, and studies which did not pay attention to this difference produced much less impressive results^{4,5,25}.

Regarding, finally, the excellent idea of Iversen *et al.* that in schizophrenia research "future effort needs to be directed to understanding of the nature of the relationship between dopamine and other modulatory neuronal systems in the brain", a whole section in my article in fact dealt with exactly this topic, namely the possible interrelationship between the dopamine and the noradrenaline systems in the brain and shows why I think that the involvement of noradrenaline in schizophrenia deserves serious consideration.

In conclusion, there is, on careful examination, less contradiction in reports on brain noradrenaline changes in schizophrenia, or at least its paranoid variety, than there is in reports on dopamine in schizophrenia. At present, therefore, the direct neurochemical evidence for abnormal noradrenaline in schizophrenic brain looks better than any such evidence for a direct brain dopamine disturbance. This is why I find the case of brain noradrenaline more convincing than the case of brain dopamine; which does not mean that future research could not change my view in favour of a more relevant disturbance in some other brain neurotransmit-

ter system or systems, dopamine not excluded. □

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Astronomy

IRAS circular 3

THE goal of issuing circulars biweekly has proved to be more difficult to achieve than we had expected. The source-selection algorithms have undergone several revisions, but they are still not sufficiently well refined to cope with the complexity that we observe in the sky. We believe, however, that this and subsequent circulars will be significantly more reliable than the first two.

It was discovered that the source listed in circular no.2 (*News and Views* **304**, 218; 1983) as 0320+613P02 had been reconstructed by the data-processing system out of several nearby sources. Thus the flux densities quoted are meaningless, and the entry should be disregarded.

This circular presents the observed positions and flux densities of 43 sources. □

G. Neugebauer and H. Habing

The source name consists of four parts: (1) the letters 'IRAS' to indicate the origin; (2) the right ascension (RA) in hours and minutes, seconds omitted; (3) declination (Dec) in decimal degrees, multiplied by 10 and then truncated (thus +32° 42.3 min becomes +327); (4) an appendix starting with 'P' and followed by the number of the circular; this appendix stresses that the data are preliminary. Position is given at Equinox 1950.0. The measurements have been made between Epochs 1983.1 and 1983.3. Wavelengths (μm): band 1: centre 12, width 6; band 2: centre 25, width 11; band 3: centre 60, width 40; band 4: centre 100, width 37.

Source IRAS	RA (1950) h min s	Dec (1950) deg arc min	12 μm	25 μm	60 μm	100 μm	Source IRAS	RA (1950) h min s	Dec (1950) deg arc min	12 μm	25 μm	60 μm	100 μm
0125+847P0	01 25 28	+84 45.2	<0.2	<0.2	0.50	2.3	0445+513P0	04 45 32	+51 19.2	6.8	7.4	2.6	<6.0
0344+728P0	03 44 59	+72 52.7	<0.4	0.69	6.0	14.0	0447+428P0	04 47 42	+42 48.9	<0.8	0.62	7.0	14.0
0356+216P0	03 56 33	+21 39.3	<0.2	<0.2	0.76	1.9	0448+445P0	04 48 09	+44 31.0	<0.4	0.55	6.0	11.0
0402+212P0	04 02 19	+21 14.3	<0.2	<0.2	1.2	2.6	0453+444P0	04 53 05	+44 28.0	87.0	89.0	23.0	8.4
0406+085P0	04 06 30	+08 31.1	<0.2	<0.2	0.95	4.8	0500-030P0	05 00 46	-03 00.4	<0.2	<0.3	3.3	8.4
0409+054P0	04 09 42	+05 25.1	0.55	0.79	9.3	20.8	0503-100P0	05 03 35	-10 03.0	<0.4	0.30	2.4	5.3
0413+081P0	04 13 24	+08 03.5	<0.2	0.68	5.4	7.0	0504-063P0	05 04 40	-06 22.7	<0.2	<0.2	3.1	<1.0
0414+011P0	04 14 07	+01 03.6	<0.2	<0.2	0.77	2.3	0504+442P0	05 04 51	+44 16.9	<0.2	0.64	5.0	12.0
0414+001P0	04 14 11	+00 09.0	<0.2	<0.4	2.0	4.0	0508-094P0	05 08 45	-09 27.0	<0.3	<0.3	3.0	7.7
0414+103P0	04 14 29	+10 20.1	<0.2	0.30	3.1	8.5	0509-204P0	05 09 29	-20 29.2	<0.2	0.63	4.2	9.2
0416+031P0	04 16 13	+03 06.6	<0.2	<0.2	0.75	2.9	0509-151P0	05 09 30	-15 11.7	<0.4	0.39	3.0	5.9
0417+751P0	04 17 03	+75 10.7	0.41	0.89	10.0	31.0	0509-157P0	05 09 48	-15 44.8	0.39	0.64	7.8	23.0
0423+536P0	04 23 50	+53 36.4	0.67	1.4	11.0	30.0	0510-244P0	05 10 05	-24 25.5	<0.2	0.40	4.7	7.3
0425+695P0	04 25 40	+69 30.2	3.7	3.9	<0.5	<3.0	0511-106P0	05 11 44	-10 41.0	<0.2	<0.2	2.9	7.9
0426+523P0	04 26 45	+52 20.6	<0.3	<1.0	12.0	100.0	0514-124P0	05 14 26	-12 24.2	<0.2	0.51	3.7	6.6
0432+476P0	04 32 15	+47 36.9	<0.4	0.56	5.1	15.0	0514-238P0	05 14 33	-23 50.5	<0.2	0.31	2.3	4.4
0433+438P0	04 33 31	+43 49.6	<0.3	0.69	8.3	21.0	0517-180P0	05 17 20	-18 02.5	<0.3	<0.3	2.3	3.8
0433+605P0	04 33 39	+60 34.1	<0.6	<0.3	4.5	7.8	0517-184P0	05 17 33	-18 27.6	2.1	2.5	<0.3	<1.0
0434+485P0	04 34 31	+48 35.7	0.46	1.4	13.0	23.0	0519-262P0	05 19 28	-26 17.2	<0.2	<0.2	1.8	3.8
0435+676P0	04 35 40	+67 38.3	<0.2	1.6	5.8	7.1	0524-218P0	05 24 07	-21 53.4	<0.2	0.26	2.3	6.5
0436+492P0	04 36 15	+49 13.0	0.46	1.3	5.5	24.0	1835+387P0	18 35 15	+38 44.2	28.0	8.7	11.8	7.1
0438+573P0	04 38 36	+57 22.1	17.0	24.0	5.1	<3.0							

Particle physics

Do metals conduct colour charge?

from Frank Close

Do quarks in complex nuclei behave in the same way as when they are in free protons or can they leak out of individual nucleons and voyage across the nucleus? Are heavy nuclei such as iron made of simply protons and neutrons, or are other components present? Do low-energy probes give an incomplete picture of nuclei and if so, in what way must we revise or extend that picture to understand the response of nuclei to high-energy probes?

These are the sorts of question being asked as a result of an astonishing effect detected in experiments at CERN, Geneva, involving scattering of high-energy muons (for practical purposes analogous to electrons) from iron. The experiments were performed by a group known as the European Muon Collaboration (EMC) and the effect named the 'EMC effect'.

In the early 1970s, high-energy electromagnetic interactions were studied in the US at Stanford, and in Europe at the electron synchrotron in Hamburg, and the Daresbury Laboratory. By 1977 high-energy physics experiments had ended at Daresbury as part of the cost of supporting European collaboration at the then new CERN super proton synchrotron — a machine which would enable electromagnetic interactions to be studied using muon beams at energies far higher than elsewhere in the world. The British group combined with their rivals on the Hamburg machine to form the EMC.

Their expertise combined with the quality of the machine has made it possible to obtain beautiful data for scattering on nuclear targets which have broken ground inaccessible to the lower-energy machine at Stanford Linear Accelerator Center (SLAC). This has proved seminal in investigating the nature of the forces acting on quarks and helping establish the quantum chromodynamic theories of quark forces. But it is when data on heavy nuclear targets, in particular iron, were compared with light deuterium data that the unexpected occurred.

The behaviour of the muon scattering on iron can be compared with that from protons and neutrons (deuterium). In such experiments the energy transferred to the nucleus and the momentum of the nuclear recoil can be independently controlled. When the momentum and energy transferred from the muons to the target are comparable (in units $\hbar = c = 1$), some effects due to proton or neutron motion within the iron nucleus are expected and are seen. But when the energy transferred to the target is large compared with the momentum, the responses of iron

and deuterium were expected to be rather similar (apart from a trivial increase in reaction probability because iron has 28 times as many nucleons as deuterium). To their surprise, the group found an extra energy-dependent component in the scattering from iron over and above that expected intuitively. The contribution is about 10 per cent of the total cross-section in the relevant kinematic region.

The group first noticed this phenomenon in 1980 and were initially cautious. As data accumulated they painstakingly searched for ways of explaining it as some overlooked systematic effect in the experimental conditions rather than a genuine natural phenomenon. They are now convinced that the effect is real and have published it (J. J. Aubert *et al.* *Phys. Lett.* **123B**, 275; 1983).

Over the same years analogous experiments were being performed at lower energy at SLAC. Back in 1972 electrons were scattered from liquid hydrogen and deuterium targets in metal containers. Sometimes electrons scattered from the liquid, sometimes from the container, so data were also taken with the container empty. A. Bodek, D. Coward and J. Friedman realized that the 'background' data obtained with an empty container could be used to see whether it showed effects similar to those seen at CERN. But the SLAC computer has undergone many changes in the last 10 years, and the data tape could no longer be read there. Fortunately the computer at Argonne near Chicago still has some suitable disc drive units and the 1972 data were finally regenerated. The comparison between their steel and deuterium data shows an effect like that seen at CERN in the region of kinematic overlap.

So the effect does indeed appear to be real. But what exactly does it mean?

One possibility, suggested by C. Llewellyn Smith at Oxford University and by M. Ericson and A. Thomas at CERN is that virtual pions surrounding the nucleons and responsible for the nuclear binding may be a more important component of heavy nuclei than had been previously assumed by most physicists. If so, this would give an effect that, with suitable choice of parameters, is qualitatively similar to that observed in the experiments.

Perhaps more exciting is the possibility that the behaviour of quarks in a complex nucleus is not simply equal to that in free nucleons. Historically it has been assumed that quarks are confined in nucleons and are unaffected by the external environment. This cannot be exactly true because

neighbouring nucleons in a nucleus can exchange quarks, thereby modifying the momentum distribution of quarks in the free nucleons (analogous to the way electrons in molecules have different overall behaviour from that in free atoms). R. Jaffe at MIT has suggested that colour charges on quarks, which are neutralized when three quarks attract one another, might within a nucleus form a colour ion, effectively being neutralized only in multiples of six rather than in two sets of three. This possibility has been recognized for some time — there have even been proposals that new stable nuclei might exist (*Nature* **296**, 305; 1982). Jaffe's proposal is not that extreme, he merely suggests that some fraction of the iron nucleus might contain six-quark clusters rather than separate three-quark nucleons. If he is right then conventional three-quark clusters coalesce at normal nuclear densities. This result could have exciting implications for the possibility that quark-gluon plasmas might be formed in heavy-ion collisions; it is conjectured that these might occur at high densities, but the EMC effect might imply that lower densities are in fact sufficient.

The idea that quark distributions are distorted in nuclei has been pursued by F. Close, R. Roberts and G. Ross at the Rutherford Laboratory in the UK. This group noticed that the EMC effect almost disappears if deuterium data at some chosen momentum transfer are compared with iron data, not at the same momentum transfer, but instead at half the momentum transfer. If one can remove the effect empirically by changing the momentum scale, then these authors suggest that conversely the effect's origins are due to some essential change of energy, or equivalently length, scale in going from deuterium to iron targets. At first sight this is no surprise since we know that there is an essential change of scale: iron and deuterium have different sizes. But the muon beams are interacting with the quarks, and it is conventionally believed that quarks are confined inside nucleons in which case the size of the nucleus has little to do with anything. So the question remains: what is the essential change of scale that is controlling the phenomenon?

Notwithstanding the comments above, Nachtmann and Pirner at Heidelberg have made the radical suggestion that as muons probe with increasing momentum there is a transition in the nuclei from colour insulation (free quark clusters) to colour conductivity (nuclei consist of uncorrelated quarks). They claim that quarks could propagate freely throughout the whole nuclear volume in which case the change of length scale is indeed that of the nuclear dimension. The Rutherford group, by contrast, have attempted to make a quantitative investigation of the possibility of a length-scale change by using the standard quantum chromodynamic theory of quark forces and seeing what effects

might be expected if one changed the scale of quark confinement. In the conventional folklore the forces between the quarks grow as the quarks are pulled apart and these forces become infinite in strength when quarks are separated by about one fermi — hence the one-fermi size of a free nucleon. What these authors find is that if the quarks can be separated by a further 10–20 per cent distance within a nucleus than in a free nucleon, then quantitatively an effect similar to that observed by the EMC obtains. Such a modest ‘deconfinement’ might not be unreasonable and might even be a fundamental underwriting of the enhanced pion cloud interpretation of Llewellyn-Smith (at quark level the enhanced pion cloud might be interpreted as a ‘leaking’ of the quarks between neighbouring nucleons).

The challenge for theorists now is to decide whether this phenomenon is indeed the first evidence for some radical effects at the quark level or whether its interpretation is more mundane. Analogous experiments have been performed at CERN and in the US using neutrino beams in place of muons. The first results were announced at the European Physical Society conference in Brighton in July. There is some controversy in the interpretation but data are consistent with the effect. Scattering experiments using electron beams have been carried out at Stanford on various target nuclei ranging across the periodic table. Again, preliminary indications at the Sussex meeting were that the effect is being seen. Only as further information accumulates will it become clear as to how fundamental this effect is — whether its origin is in ‘old-fashioned’ nuclear physics, or whether it is giving radical information about the behaviour of quarks and colour forces. □

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Messrs Siemens and Halske have brought out an instrument called a torsion galvanometer to be used for large currents. It consists of a magnet suspended between two coils, so as to be affected by both, but to which is attached a torsion spring so arranged that the amount of torsion necessary to bring the needle back to its normal position can easily be determined. These instruments are made in two forms, a vertical and a horizontal form. In the vertical form the needle is suspended by a cocoon silk, and the reading is taken from above; this is the more delicate form. In the horizontal form, which is meant for more practical work, the needle is balanced on knife-edges, and carries at one end a light pointer which passes behind a scale. The amount of torsion required to bring the needle back to zero is indicated by another pointer attached to a handle, and which moves in front of the scale.

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Atomic spectroscopy

Tricks for lasers and atom beams

from Anne Thorne

NEUTRAL atoms can be steered, focused, scattered or trapped by laser beams, just as ions can be manipulated by suitable configurations of electric and magnetic fields. Beams of neutral atoms and ions alike can be heated or cooled by transfer of photon momentum from collinear laser beams. These effects have been investigated for some years; but, as E. Arimondo (University of Naples) explained at the 15th EGAS Conference in Madrid*, it is necessary to consider fluctuations and the effects of spontaneous emission to describe what really happens.

If, for example, a laser tuned to the short-wavelength side of resonance is directed against an atomic beam, the faster atoms absorb photons and correspondingly lose momentum, but the reduction in both velocity and velocity spread in the beam direction is accompanied by an increase in spread (that is, laser heating) perpendicular to the axis as a result of the spontaneous emission. On the other hand, the collimation of the beam can be increased by the so-called gradient force. This is just the optical analogue of the Stern–Gerlach effect: the induced electric dipole resulting from saturation of the resonant atomic transition interacts with the radial intensity gradient of the laser beam to give an inward or outward radial force according to whether the laser is tuned to the long- or short-wavelength side of resonance. This force is the basis for optical trapping of neutral atoms — which has in fact not yet been realized. The importance of fluctuations in the absorption-stimulated emission is best seen when an atomic beam is directed perpendicularly to the standing wave pattern that results from counter-propagating laser beams. Here the average net force is zero, but the fluctuations give rise to scattering in the transverse direction that can well be described as ‘Bragg diffraction from a laser crystal’.

The use of narrow-band tunable dye lasers for high-resolution spectroscopy and lifetime measurements is of course well established. R. Hallin (University of Uppsala) surveyed some of the tricks that can be played by exciting ions in fast beams from accelerators. For example, with a laser collinear with a 50-KeV beam, the Doppler width for two photon excitation can be reduced to a few megahertz (a resolving power of order 10^8), and it is possible to tune to an intermediate resonance by adjusting the energy of the beam. Applied to lifetime measurements, the fast-beam

techniques can have a time resolution approaching 10^{-10} seconds. Several of the contributed papers at the meeting presented specific applications of these techniques.

Another interesting combination of lasers with monoenergetic atomic beams leads to determinations of the differential cross-sections for inelastic collisions. A change in relative velocity from such a collision is manifested as a Doppler shift, detectable by scanning a laser across an absorption line starting from the final state.

The contributed papers covered the usual wide range of experimental and theoretical work on energy levels and term analysis, transition probabilities, hyperfine and isotope structure, collisional and relativistic effects. Among topics that can, perhaps, be considered a little more unusual is the laser heating effect resulting from collisionally aided radiative excitation. In this process the excess photon energy from a laser tuned to the high-frequency side of a transition in atom A is converted to translational energy shared between A and a perturbing atom B. The resulting change in Doppler width of A with different perturbers B has been measured; evidently this effect could lead to macroscopic local heating (or cooling) of the gas.

A number of contributions were concerned with the effects of high external fields on both bound states of atoms and the photoionization continuum — the quadratic Zeeman effect, quasi-Landau resonances and so on. An interesting alternative, depending on the availability of very high spectral resolution in the form of narrow-band lasers and Doppler-free methods, is to work at such high principal quantum numbers that relatively modest fields produce the same effects, a few hundred gauss or a few volts per centimetre at $n \sim 200$, for example. The group at Ecole Normale Supérieure, Paris, conducting these high- n experiments has also demonstrated linear Stark effect in their quasi-hydrogenic high- n states of caesium, an effect originally predicted in 1926.

To end on a historical note, a mention of the 1920s in conjunction with Madrid brings to mind the discovery of complex spectra by Catalan. By a happy coincidence the two senior spectroscopists at the Madrid meeting were B. Edlen from Lund and W. Garton from Imperial College, London, the two laboratories with which Catalan collaborated in his classic work. □

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*The 15th European Group for Atomic Spectroscopy Conference was held in Madrid, 5–8 July, 1983.

Biochemistry

Dynamic proteins

from Roger H. Pain

THERE is no shadow of doubt that enzymes are not the rigid molecules that their traditional models necessarily suggest. The internal packing density of atoms, apparent from X-ray crystallography, is sufficiently low to allow a degree of motion within folded protein molecules¹. As a result small molecules such as water², acrylamide³ and oxygen⁴ can migrate through the interior with remarkable ease, occupying 'mobile defects' generated by thermal motion of the atoms⁵. Atomic motions permit asymmetric aromatic groups elbow room to flip rapidly (2–3 ps) through 180° every 10⁻⁴ s or so⁶. Even X-ray crystallographers, goaded by the experiments and hypotheses of solution biochemists, have found evidence for motion in the temperature-dependent blurring of electron density around atoms in the crystalline state⁷. The dynamics of protein conformation range over a broad spectrum from picoseconds to longer than milliseconds, from the rotation about carbon-carbon bonds in side chains to the bending of whole domains about molecular hinges^{8,9}.

Sufficient clues have now accumulated to suggest that dynamic properties are crucial to biological function. Binding of a ligand can have a marked effect on the internal mobility of an enzyme^{10,11} even though, in the case of lysozyme, the change in the mean atomic positions is relatively small. The complete understanding of binding constants and, by inference, of allosteric phenomena must therefore depend on knowledge of the dynamics and their enthalpic and entropic implications. The binding of oxygen to myoglobin involves its diffusion through an apparently closely packed region¹². The distinction between inactive zymogen and active enzyme is not so much a matter of different active-site geometry but of altered dynamics¹³. The delicate balance of stability which is needed to define the appropriate rate of protein turnover *in vivo* will depend on the nature and extent of the vibrational excursions of the molecule. Moreover, there have been suggestions that the native state of an enzyme may be modified by ligands to exhibit differing catalytic activities¹⁴ or, to put it another way, that the native state comprises a collection of conformers in equilibrium with each other, each with differing biological activity and able to be selected by a different ligand¹⁵.

To understand the biological function of proteins at the molecular level clearly demands a detailed understanding of the nature and energetics of the dynamics and also a new kind of modelling process which will make it possible to visualize and handle the vast amount of information that will be generated. McCammon, Gelin and

Karplus¹⁶ broke new ground in applying the method of molecular dynamics to simulate the motion of protein molecules. This involves the daunting task of solving, numerically, the equations of motion for all the atoms in the molecule at the same time, at time intervals of 2×10^{-15} s over as long a period of time as computing funds will allow. Such a computation will lead to trajectories for all the atoms in the molecule which will, in principle, define the state of the molecule over that time period. Results of similar calculations were made more accessible in a computer film depicting the motion of a space-filling model of pancreatic trypsin inhibitor over a time period of a fraction of a nanosecond¹⁷.

The audience response to the molecule swallowing (or expectorating, depending on which way the film is seen) is impressive. As a visual aid to understanding protein dynamics the method is unparalleled. The question is, how closely do the results correspond to reality? The criteria are two-fold, static and dynamic. First, the mean position of each atom in the protein, averaged over all its excursions over a substantial period of time, should correspond to the mean position determined by X-ray crystallography at high resolution. Implicit in this is the assumption that the contacts between molecules in the crystal will not substantially modify the dynamics and the mean atomic positions, compared with the isolated molecule which the computer can handle. Second, the motions predicted by molecular dynamics for the whole molecule should correlate with experimental observations of the motions of limited parts of the molecule by electron density, temperature factors, nuclear magnetic resonance, fluorescence and so on.

The problems are formidable. There is no common agreement on the potential energy functions for the interactions encountered, such as hydrogen bonds, hydrophobic and solvent interactions, electrostatic interactions involving a dielectric 'constant'. The computing time required is considerable — 140 CPU hours were used on a Cyber 170/760 in a recent simulation over 20 ps (ref. 18) — with the consequence that simplifications and short cuts have to be balanced against size of protein and time of trajectory. While subnanosecond times are becoming accessible for small proteins, the interesting fluctuations which occur with longer lifetimes are barely on the horizon.

Two recent groups of papers illustrate the progress which is being made in the field. Those by Levitt¹⁹ are lengthy but clear expositions, giving the layman a feel

for the methods, problems and the achievements in terms of a detailed description of the dynamics of pancreatic trypsin inhibitor. Following the general pattern of initial equilibration of the experimental protein structure to an energy minimum, 'warming up' to the temperature of operation, and then carrying out the molecular dynamics computation, he shows the advantages of an extensive period of equilibration to eliminate initial strain in the structure. How much the latter reflects imperfection in the crystal structure and how much in the energy functions used in the simulation would be interesting to know.

Levitt studied the isolated molecule in the absence of bulk solvent which allowed generation of a 132-ps trajectory. The main feature is the choice of a hydrogen-bond function which strongly favours linear bonds as opposed to the more conventional electrostatic interaction. This led to better agreement of the simulated and X-ray structures and has the advantage of not restricting conformational freedom during the simulation by prior listing of hydrogen-bond interactions. The most interesting feature, perhaps because it accords with prior prejudices about conformational mobility, is that during the 132-ps trajectory the protein undergoes minor transitions between four identifiable and persistent (over 20–30 ps) conformations. One of these transitions is to a large extent reversible. The conformations differ appreciably in the balance between energy contributions from hydrogen bonds, torsion angles and van der Waals' interactions. It is not unlikely that these or similar conformations would be distinguishable by immunological probes and would be sufficient to have catalytic consequences for an enzyme.

The other paper¹⁸, also on pancreatic trypsin inhibitor, is complementary to

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those of Levitt. Van Gunsteren *et al.* use the non-specific electrostatic and van der Waals' interaction function of Lifson for their hydrogen bonds. They attempt, however, to take account of the inter-molecular contacts and solvent environment on the dynamics of a protein in the crystal in order to improve the agreement with the X-ray structure. Their simulation was carried out on a unit cell containing four protein molecules and 560 water molecules with the result that the time span was limited to a 20-ps trajectory. The structure of each molecule differs from the other three and from the X-ray structure, but the

average over all four differs from the X-ray structure by only 1.2 Å r.m.s. for all atoms in the molecule.

All in all, results from computer simulation well justify Weber's²⁰ statement: "The protein molecule model resulting from the X-ray crystallographic observations is a 'platonic' protein, well removed in its perfection from the kicking and screaming 'stochastic' molecule that we infer must exist in solution". □

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Planetary science

Magnetism and evolution of the terrestrial planets

from J.A. Jacobs

A RECENT paper by David Stevenson and his colleagues¹ attempts to account for the properties (or absence) of intrinsic magnetic fields in the terrestrial planets — Earth, Mercury, Venus and Mars — in terms of their composition, internal structure and thermal history. Except in the case of Earth, where a large amount of seismic data is available, study of the intrinsic magnetic field provides one of the few clues to the nature of a planet's interior. Although Stevenson *et al.* cannot come to firm conclusions for all the planets, their analysis makes it possible to rule out a number of suggested models.

The Earth's magnetic field is predominantly dipolar (moment 8×10^{22} Am²), Mercury has a considerably smaller but nevertheless significant intrinsic magnetic field (dipole moment $\sim 2.8\text{--}4.9 \times 10^{19}$ Am²), whilst Venus and Mars have no, or at least extremely small, magnetic fields. The absence of a magnetic field on Venus, which is almost the same size as the Earth, cannot be because of its much slower rate of rotation (~ 243 days), since, as Hide² first pointed out, Coriolis effects are still dominant for any large-scale motions in the core.

It is generally accepted that the Earth's magnetic field is produced by dynamo action in the mainly iron fluid outer core, although there is not complete agreement on what drives the fluid motions. The most probable cause is either thermal convection or chemical buoyancy due to gravitational differentiation of the core into a solid heavier inner core and a lighter fluid outer core. The main advantage of chemically driven convection is its much greater thermodynamic efficiency. Indeed, convection driven by gravitational differentiation may occur even though the overall temperature gradient is sub-adiabatic³. In their analysis, Stevenson *et al.* assume that all the terrestrial planets underwent differentiation into a core and mantle and that any

magnetic field is the result of dynamo action driven by thermal or chemical convection in a fluid core or fluid outer core.

If substantial radioactive heat sources are absent in the cores of the terrestrial planets, as is now generally believed to be the case, the paper shows that the heat flux from the cores due to secular cooling alone would probably now be sub-adiabatic and dynamo action would have ceased more than 1,000 Myr ago. On the other hand, if chemical buoyancy is the driving force for the fluid motions, then the existence or non-existence of a magnetic field depends on whether the planets possess solid inner cores.

The planets are described by a two-layer model with average densities and average heat capacities for mantle and core, and material parameters chosen so that present-day estimates of heat flux, upper-mantle temperature and viscosity, and inner core radius are obtained for the Earth. Whole-mantle convection is assumed and any phase changes in the mantle neglected. The inner core is assumed to consist of pure iron, the outer core containing some light alloying component (sulphur or oxygen). Initially the whole core is super liquidus. As the planet cools, a solid inner core will begin to form when the liquidus temperature is reached at the centre; with further cooling the inner core grows at the expense of the outer core.

The validity of all the models does not depend on a precise knowledge of the melting curve of pure iron since they are adjusted to give the correct size of the inner core for the present Earth. Also, the beginning of inner core formation depends on the ability of the mantle to remove heat from the core and not on the details of the core liquidus. Small changes in model parameters can lead to completely fluid non-convecting cores, convecting fluid outer cores with inner core growth and almost completely solid cores with only a

thin outer fluid shell.

In the case of the Earth, all models give growth of an inner core beginning after 2,300–3,000 Myr, quite late in the Earth's history. Since the Earth's magnetic field is at least 3,500 Myr old (ref. 4), the driving mechanism of the geodynamo may have changed over geological time. Stevenson *et al.* suggest that initially the Earth's magnetic field was sustained by thermal convection. After inner core growth began (1,500–2,500 Myr ago), the release of gravitational energy became the dominant source for the geodynamo. The authors further suggest that this change in the energy source might be reflected in certain features of the geomagnetic field such as the frequency of polarity reversals.

The models for Venus admit all three possible evolutions. Since models with almost completely solid cores require implausibly small amounts of light alloying components, Stevenson *et al.* favour a completely fluid stably stratified core to explain the absence of a magnetic field on Venus. Two possible implications are that Venus once had an appreciable magnetic field driven by thermal convection which died $\sim 1,500$ Myr ago, and that Venus will eventually have a solid inner core which might cause the dynamo to start up again. The failure of some of the models of Venus to form an inner core, as do all Earth models, is mainly due to the lower central pressure of Venus (290 GPa compared with 360 GPa in the case of Earth).

The models for Mars also admit all three possible evolutions. Stevenson *et al.* again favour a completely fluid core since, apart from the absence of a substantial magnetic field, it is predicted for a cosmochemically plausible sulphur content of 15 per cent or more by weight. In all the models of Mercury, growth of a large solid inner core begins early in its history (after 250–600 Myr). The heat flux from the core becomes sub-adiabatic at different times for the models, but convection in a thin outer core (and hence a magnetic field) is still present in all models being maintained by chemical buoyancy.

Stevenson *et al.*'s analysis adds considerably to our knowledge of the terrestrial planets' possible constitution. Hide⁵ had already shown how to locate the electrically conducting fluid core of a planet from external magnetic observations, although in the case of the Earth not so accurately as using seismic data. There is no doubt that magnetism will continue to play an ever increasing part in helping to unravel the deeper structure of the planets. □

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Protein phosphorylation in the brain

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Protein phosphorylation represents an approach, sometimes the only approach available, to study the molecular basis for a wide variety of neurophysiological phenomena. The injection of protein kinases or protein kinase inhibitors into neurones has provided direct evidence that activation of protein kinases has an obligatory role in the mechanisms by which numerous extracellular signals produce specific physiological responses in neurones. A diversity of substrate proteins for the kinases have already been found. In several instances, the identity and functional role of these substrate proteins have been established.

A MAJOR interest of biomedical research for many years has been the elucidation of the molecular mechanisms by which hormones, neurotransmitters and other extracellular signals produce biological responses in specific target cells. A wide variety of extracellular signals, both inside and outside the nervous system, produce many of their diverse metabolic and physiological responses by regulating the state of phosphorylation of specific substrate proteins in target tissues. The large number of individual molecular pathways involving protein phosphorylation that have been elucidated in animal tissues over the past several years (see ref. 1 for review) supports the view^{1,2} that protein phosphorylation is a final common pathway of paramount importance in animal cells. This review summarizes recent evidence for such a vital role of protein phosphorylation in neuronal function.

Phosphorylation systems in nervous tissue

Individual steps in the molecular pathway by which protein phosphorylation mediates certain of the effects of extracellular signals on neuronal function are shown in Fig. 1. Extracellular signals, or first messengers, in the nervous system include a variety of neurotransmitters and hormones, as well as the nerve impulse itself. These first messengers produce many of their biological responses by regulating the intracellular concentrations of cyclic AMP, cyclic GMP or calcium in specific target neurones; these three molecules can, therefore, be considered as second messengers in neurones.

Much evidence suggests that most and possibly all of the second messenger actions on neuronal function of cyclic AMP and cyclic GMP, as well as many of the second messenger actions of calcium, are achieved through the activation of specific cyclic AMP-dependent, cyclic GMP-dependent and calcium-dependent protein kinases. The brain contains virtually one type of cyclic AMP-dependent protein kinase and one type of cyclic GMP-dependent protein kinase, but has multiple types of calcium-dependent protein kinases which fall into two subclasses. One subclass, activated in conjunction with the calcium-binding protein calmodulin, is referred to as calcium/calmodulin-dependent protein kinase. The other subclass, activated in conjunction with phosphatidylserine and other lipids, is referred to as calcium/phosphatidylserine-

dependent protein kinase. To date, four types of calcium/calmodulin-dependent protein kinases³⁻⁷ and one type of calcium/phosphatidylserine-dependent protein kinase⁸ have been found in brain. Several unanswered questions central to an understanding of calcium-dependent protein phosphorylation are: (1) How many subclasses of calcium-dependent protein kinases exist? (2) How many types of enzyme exist within each subclass? (3) What is the physiological substrate specificity of each enzyme? (4) Which calcium-dependent protein kinase(s) is responsible for each of the physiological actions of calcium that are mediated through protein phosphorylation?

Activation of these various protein kinases results in the phosphorylation of specific substrate proteins, leading, through one or more steps, to the production of specific biological responses. Three questions central to an understanding of the role of substrate proteins as intracellular signals in neurones are: (1) Which substrate proteins produce biological responses directly, and which act indirectly through intermediate steps? (2) To what extent are the interactions (synergism, antagonism) between the biological effects of two extracellular signals achieved through the phosphorylation of the same substrate protein? (3) Are the effects of substrate protein phosphorylation mediatory or modulatory with respect to the cellular process being regulated? With regard to the last question, for example, phosphorylation of ion channels may have either a mediatory role by being an obligatory step in the sequence of events through which those channels open or close in response to a first messenger, or a modulatory role by altering the sensitivity of the channels to open or close in response to other first messengers.

Protein phosphorylation may be involved in carrying out or regulating such diverse processes as neurotransmitter biosynthesis, axoplasmic transport, neurotransmitter release, generation of postsynaptic potentials, ion channel conductance, neuronal shape and motility, elaboration of dendritic and axonal processes, and development and maintenance of differentiated characteristics of neurones (see ref. 1). Some of the effects of protein phosphorylation on neuronal function may include biochemical events that underlie short-term and long-term memory. The biochemical basis of short-term memory may involve the phosphorylation of presynaptic or postsynaptic proteins in response to synaptic activity, which would result in transient facilitation or inhibition of synaptic transmission^{9,10}. The biochemical basis of long-term memory may involve the phosphorylation of proteins that play a part in the regulation

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Table 1 Systems in which direct evidence has been obtained for a role of protein phosphorylation in neuronal function

Cell (Genus)	Kinase injection	Inhibitor injection	Conclusion of studies	Ref.
Bag cell neurones (<i>Aplysia</i>)	+*	+†	Kinase mediates effect of synaptic activation and of exogenous cyclic AMP in producing the afterdischarge; this action appears to be achieved through decreases in the conductance of calcium-dependent K ⁺ channels and of early (<i>I_A</i>) voltage-dependent K ⁺ channels	11‡
Sensory neurones (<i>Aplysia</i>)	+*	+†	Kinase mediates effect of synaptic activation and of exogenous serotonin and cyclic AMP in facilitating neurotransmitter release in response to nerve impulses; this action appears to be achieved through decreases in the conductance of novel serotonin-regulated K ⁺ channels§	12,13, 57
Neurone R15 (<i>Aplysia</i>)	—	+†	Kinase mediates effect of exogenous serotonin and cyclic AMP in inhibiting bursting activity and in enhancing interburst hyperpolarization; this action seems to be achieved through increases in the conductance of novel serotonin-regulated, anomalously rectifying K ⁺ channels	14, 58
Unidentified neurones (<i>Helix</i>)	+*	—	Kinase increases conductance of calcium-dependent K ⁺ channels	15
Photoreceptor cells (<i>Hermisenda</i>)	+*	—	Kinase decreases conductance of early (<i>I_A</i>) and late (<i>I_B</i>) voltage-dependent K ⁺ channels	16
Photoreceptor cells (<i>Bufo</i>)	+	—	Kinase mediates effect of dark and of exogenous cyclic GMP in producing depolarization of rod outer segment membranes; this action seems to be achieved through increases in the conductance of Na ⁺ channels	19

* Catalytic subunit of cyclic AMP-dependent protein kinase.

† Specific inhibitor of cyclic AMP-dependent protein kinase.

‡ Also L. Kaczmarek, personal communication.

§ It has been hypothesized¹ that cyclic AMP-dependent protein kinase also facilitates neurotransmitter release from these nerve terminals through an additional mechanism involving the phosphorylation of a synaptic vesicle-associated protein, homologous to synapsin I in mammalian neurones.

|| Holoenzyme of cyclic GMP-dependent protein kinase.

of gene expression, which would result in more permanent modifications of synaptic transmission⁹.

Protein kinases regulate neuronal function

Recent studies have provided direct evidence for a causal relationship between cyclic AMP-dependent protein phosphorylation and physiological responses in excitable cells^{11–16}. These studies, summarized in Table 1, have shown that the intracellular application of the catalytic subunit of cyclic AMP-dependent protein kinase mimics specific physiological responses in a variety of molluscan neurones and that the intracellular application of an inhibitor of the catalytic subunit blocks the responses. The demonstration that a protein kinase can mimic a physiological response to a hormone or neurotransmitter indicates that activation of the protein kinase is sufficient to produce that physiological response. The demonstration that a protein kinase inhibitor prevents a physiological response to a hormone or neurotransmitter indicates that activation of the protein kinase is necessary to produce that physiological response. Taken together, such findings show that activation of the protein kinase is both a necessary and sufficient step in the sequence of events by which first messengers produce physiological responses.

The studies summarized in Table 1 have demonstrated a role for cyclic AMP-dependent protein kinase in the physiological regulation of several types of K⁺ channels in a variety of molluscan neurones. Cyclic AMP-dependent protein kinase is also involved in the pathway by which adrenaline and cyclic AMP increase the conductance of voltage-dependent calcium channels in mammalian heart muscle cells^{17,18}. The role of cyclic AMP-dependent protein kinase in regulating ion channel function supports the view^{1,2} that, through the detection and characterization of neuronal substrate proteins for various protein kinases, a detailed understanding will be achieved of the molecular mechanisms underlying diverse types of physiological responses in neurones.

Recently, injection of cyclic GMP-dependent protein kinase holoenzyme into rod outer segments has been shown to mimic the depolarizing action of cyclic GMP on rod outer segment membranes (Table 1)¹⁹. Injection of the kinase has also been

shown to antagonize the ability of light to hyperpolarize these membranes. Conversely, it was found that application of a light flash during the depolarization caused by injection of the protein kinase immediately prevents further depolarization. These findings provide the first direct evidence for a role of cyclic GMP-dependent protein kinase in cell function.

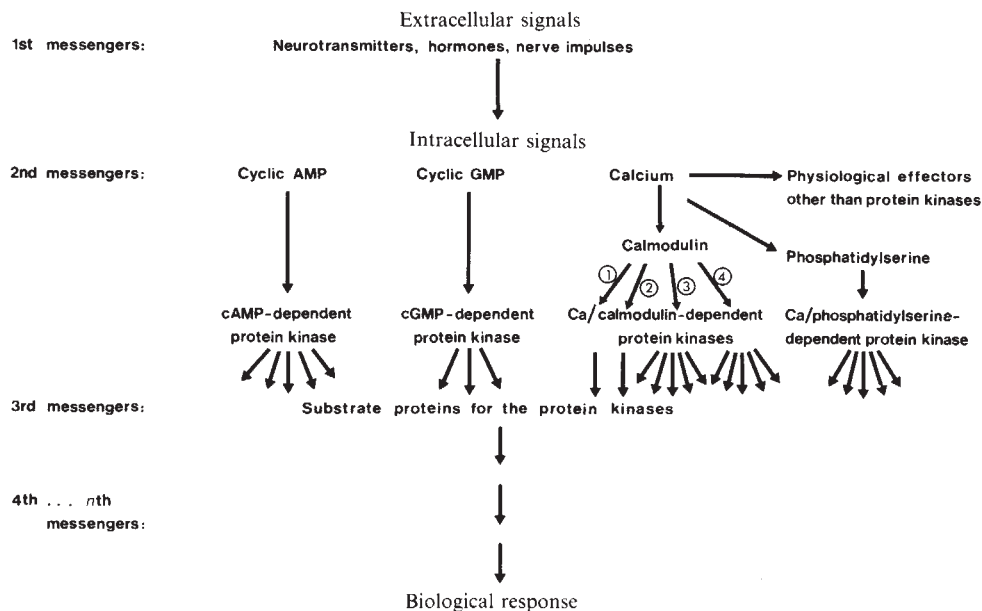
Detection of substrate proteins

Identification of the specific substrate protein(s) involved in a particular biological response is crucial to the elucidation of the molecular pathway through which that response is achieved. There are two, conceptually distinct, experimental approaches to their identification. One is to investigate the possible phosphorylation of known proteins either with established functions, such as enzymes, neurotransmitter receptors and ion channels, or those having no established function, such as cytoskeletal proteins and myelin basic protein. Unfortunately, this approach is limited to the small number of cellular proteins so far characterized, physiological processes whose molecular bases are known, and regulatory mechanisms in which known proteins are implicated.

The second approach is to search for previously unknown proteins by virtue of their phosphorylation in response to appropriate stimuli, and then to characterize these proteins with respect to their biochemical, anatomical and physiological properties. In contrast to the study of known proteins, the study of previously unknown proteins is a strikingly more open-ended approach with the potential for discovery of previously unsuspected regulatory pathways. As an example, the discovery and characterization of synapsin I (see below) have provided evidence that a protein associated with neurotransmitter vesicles may be involved in a novel mechanism that regulates neurotransmitter release. Synapsin I is one of about 70 prominent phosphoproteins which serve as substrates for cyclic AMP-dependent, cyclic GMP-dependent or calcium-dependent protein kinases, and which seem specific to nervous tissue, that have already been found in brain using this approach^{20,21}.

It is one thing to detect and characterize a protein as a phosphorylated band on an SDS-polyacrylamide gel. Elucidating the biological function of such a protein is a much more

Fig. 1 Signals in the brain. Extracellular signals (first messengers) produce specific biological responses in target neurones via a series of intracellular signals (second, third, etc. messengers). Second messengers in the brain include cyclic AMP, cyclic GMP and calcium. Cyclic AMP and cyclic GMP produce most, and possibly all, of their second messenger actions through the activation of virtually one type of cyclic AMP-dependent protein kinase and one type of cyclic GMP-dependent protein kinase, respectively. The former enzyme exhibits a broad substrate specificity and the latter a more restricted specificity. Calcium exerts many of its second messenger actions through the activation of calcium-dependent protein kinases, as well as through a variety of physiological effectors other than protein kinases. Calcium activates protein kinases in conjunction with calmodulin or phosphatidyserine. There are at least four types of calcium/calmodulin-dependent protein kinases in brain^{3-7,55,56}: (1) phosphorylase kinase which phosphorylates only phosphorylase (and possibly glycogen synthase); (2) myosin light chain kinase, which phosphorylates only myosin light chain; (3) calcium/calmodulin-dependent protein kinase I; and (4) calcium/calmodulin-dependent protein kinase II. The substrate specificities of the latter two enzymes have not been established, but both appear to phosphorylate numerous substrate proteins. The activation of individual protein kinases causes the phosphorylation of specific substrate proteins in target neurones. In some cases, these substrate proteins, or third messengers, appear to be the immediate effector for the biological response. In other cases, they seem to produce the biological response indirectly through fourth, fifth, sixth, etc. messengers.



formidable task. However, the difficulty of the task should not be confused with the legitimacy and importance of the approach as a means, and sometimes the only means, of elucidating a wide variety of molecular pathways involved in the regulation of cell function.

Characterization of substrate proteins

A novel substrate protein needs to be characterized in terms of (1) its protein kinase specificity; (2) its regional distribution in the central and peripheral nervous systems, the types of cells within such a region in which it occurs, and its subcellular localization within those cells; (3) its molecular biology, including the regulation of its expression during development and in mature neurones. In addition, the phosphorylation of the substrate protein must be studied in intact cells in order to identify the first messengers involved in the regulation of its state of phosphorylation *in vivo*. These various characterizations of the substrate protein all contribute to the formulation of a hypothesis of its functional role.

The validity of the hypothesis can be studied by introducing purified components of the protein phosphorylation system, and inhibitors of these components, into various types of neuronal preparations, and monitoring the physiological effects of such manipulations. For example, does introduction of the substrate protein, in either the phosphorylated or dephosphorylated form, into an experimental preparation either enhance or antagonize the physiological process being monitored? In addition to the substrate protein, other components that can be used in these studies include antibodies specific for the phospho- or dephospho-form of the protein²², the protein kinase(s) and protein phosphatase(s) involved in its phosphorylation and dephosphorylation, as well as activators and inhibitors of these various enzymes.

Some characterized substrate proteins

The biochemical, anatomical and physiological properties of some neuronal substrate proteins that have been characterized are summarized in Table 2. Neuronal phosphoproteins differ in a variety of their biochemical and physicochemical properties. Most are phosphorylated on serine residues; some are phos-

phorylated on threonine residues. In addition, most of the proteins are phosphorylated on multiple amino acid residues, but others are phosphorylated on only a single residue. Some of the proteins appear to be phosphorylated by only one protein kinase, whereas others are phosphorylated by more than one protein kinase. Microtubule-associated protein-2 (MAP-2) has been reported to be phosphorylated by four distinct protein kinases.

Some of the proteins are phosphorylated on more than one site by a single protein kinase—for example, rhodopsin kinase catalyses the incorporation of up to 9 mol of phosphate into rhodopsin; others, such as synapsin I and protein III, are phosphorylated on the same site by more than one protein kinase; and yet others, such as γ -aminobutyric acid (GABA)-modulin²³, MAP-2²⁴ and tyrosine hydroxylase^{25,26}, are apparently phosphorylated on distinct sites by different protein kinases. Phosphorylation of a substrate protein at the same or at distinct sites by more than one second messenger/protein phosphorylation system provides a molecular basis for the convergence of the pathways by which these second messenger systems regulate cell function (see ref. 1).

Neuronal phosphoproteins also differ in their regional, cellular and subcellular distributions. Some of the proteins seem to be present in virtually every neurone—for example, synapsin I. Others are present in only certain neuronal cell types—for example, DARPP-32 (dopamine and cyclic AMP-regulated phosphoprotein of 32,000 molecular weight) is highly enriched in dopaminergic neurones that possess D₁-dopamine receptors, and G-substrate is present in only a single type of neurone, the cerebellar Purkinje cell. The specific localization of phosphoproteins to certain neuronal cell types has made it possible to use antisera against these proteins to study various anatomical and developmental aspects of the neurones in which the proteins occur (see refs 1, 27–30). Some neuronal phosphoproteins are presynaptic, whereas others are postsynaptic, and some are cytosolic, others particulate. Among the particulate substrate proteins, some are associated with synaptic vesicles, some with cytoskeletal structures, and others with plasma membranes.

Table 2 also illustrates the wide diversity of biological functions of neuronal phosphoproteins and the mechanisms by

Table 2 Some properties of neuronal phosphoproteins

Substrate protein	M_r	Distribution: regional	Distribution: subcellular	1st messengers regulating phosphorylation	Protein kinase(s)	Phosphorylation site(s)*	Effect of phosphorylation on protein†	Ref.
Tyrosine hydroxylase	60K subunit	Catechol-aminergic neurones	Predominantly cytosol	Nerve impulses Acetylcholine	Cyclic AMP Calcium/ phosphatidylserine Calcium/ calmodulin	1 site? (cyclic AMP)	Increases catalytic activity (cyclic AMP or calcium)‡	25, 26 59-72
Nicotinic acetylcholine receptor (γ and δ subunits)	γ , 60K; δ , 65K	<i>Torpedo</i> electroplax and nicotinic cholinergic neurones	Plasma membrane		Cyclic AMP (γ and δ subunits) Calcium/ phosphatidylserine (δ subunit)	1 serine residue on each (cyclic AMP)		73-77
Voltage-dependent sodium channel (α subunit)	270K	All neurones	Plasma membrane		Cyclic AMP	Up to 4 sites?		78, 79
Rhodopsin	38K	Photoreceptors	Rod outer segment membranes	Light	Rhodopsin	Up to 9 sites?	Decreases ability of rhodopsin to activate phosphodiesterase	80-88
Microtubule-associated protein-2 (MAP-2)	300K	All neurones	Microtubules		Cyclic AMP Calcium/ phosphatidylserine Calcium/calmodulin Neurofilament	Up to 3 sites? (cyclic AMP) Up to 5 sites? (calcium/calmodulin)	Promotes assembly <i>in vitro</i> ? (cyclic AMP) Promotes disassembly <i>in vitro</i> ? (calcium/calmodulin)	21, 24 89-96
Neurofilament proteins (all subunits)	200K, 145K, 68K	All neurones	Neurofilaments		Neurofilament (all subunits) Cyclic AMP (145K subunit)	Predominantly serine (200K and 68K) Serine and threonine (145K)		92, 97-103
Myelin basic protein (small and large)	14K, 18K	Oligodendrocytes, and Schwann cells	Myelin		Calcium/ phosphatidylserine Calcium/ calmodulin? Myelin?	Predominantly serine, some threonine	Alters immunogenicity?	95, 104-113
GABA-modulin	16.5K	GABA-ceptive neurones	Synaptic membranes		Cyclic AMP Calcium/ phosphatidylserine Calcium/calmodulin	Up to 4 sites? (cyclic AMP) 1 site? (calcium/calmodulin)	Decreases ability of GABA-modulin to inhibit binding of GABA to GABA receptors (cyclic AMP)	23, 114
Synapsin I	Ia, 86K; Ib, 80K	All neurones	Synaptic vesicles	Nerve impulses (sites 1, 2, 3) Serotonin, dopamine, noradrenaline (site 1) Depolarizing agents (sites 1, 2, 3) Convulsants and depressants (?)	Cyclic AMP (site 1) Calcium/calmodulin I (site 1) Calcium/calmodulin II (sites 2 and 3)	3 serine residues (sites 1, 2 and 3)	Decreases affinity for synaptic vesicles (calcium/calmodulin II)	1, 4-7, 31-47, 115-118
Protein III	IIIa, 74K; IIIb, 55K	All neurones	Synaptic vesicles	Nerve impulses Serotonin, dopamine, noradrenaline Depolarizing agents	Cyclic AMP Calcium/ calmodulin I	1 serine residue		42, 43, 46, 116, 119, 120§
DARPP-32	32K	Dopaminergic neurones that possess D_1 receptors	Cytosol	Dopamine	Cyclic AMP	1 threonine residue	Increases potency as phosphatase inhibitor (cyclic AMP)	28, 121-123¶
G-substrate	23K	Cerebellar Purkinje cells	Cytosol	Excitatory neurotransmitters (acetylcholine, glutamate)	Cyclic GMP	2 threonine residues	Increases potency as phosphatase inhibitor (cyclic GMP)	124-129
87K	87K	All neurones?	Cytosol	Depolarizing agents	Calcium/ phosphatidylserine			130

First messengers regulate not only the state of phosphorylation, but also the total amount of substrate proteins. The total amount of tyrosine hydroxylase is increased by nerve impulses¹³¹⁻¹³³, acetylcholine¹³¹ and glucocorticoids^{134,135}. The total amount of the nicotinic acetylcholine receptor is regulated by nerve impulses^{132,136}. The total amount of synapsin I is regulated by noradrenaline¹³⁷, corticosterone¹³⁸ and morphine (K. Tsou, E. B. Per Dahl and P. G., unpublished observation). The protein kinases listed in column 6 are: cyclic AMP, cyclic AMP-dependent protein kinase; calcium/calmodulin, calcium/calmodulin-dependent protein kinase; calcium/phosphatidylserine, calcium/phosphatidylserine-dependent protein kinase; rhodopsin, rhodopsin kinase; neurofilament, neurofilament-associated protein kinase; myelin, myelin-associated protein kinase; calcium/calmodulin I and calcium/calmodulin II, calcium/calmodulin-dependent protein kinase I and II, respectively; cyclic GMP, cyclic GMP-dependent protein kinase. Rhodopsin kinase^{82,85,86}, neurofilament-associated protein kinase^{92,102,103} and myelin-associated protein kinase¹⁰⁷ are reported to be 'independent' protein kinases, as cellular messengers that activate them *in vivo* have not yet been identified. M_r , molecular weight (in thousands, K).

* The protein kinase responsible is indicated in parentheses.

† The protein kinase that produces this effect on the substrate protein is indicated in parentheses.

‡ Increased activity of tyrosine hydroxylase has been reported to be associated with an increased affinity for the pterin cofactor and for tyrosine, with a decreased affinity for end-product inhibitors, and with an increase in V_{max} .

§ Also M. Browning, C. Huang, S. I. Walaas, P. L. Mobley and P. G., unpublished observations.

¶ Also H. Hemmings, A. Nairn and P. G., unpublished observations.

which they produce physiological responses. For example, phosphorylation of tyrosine hydroxylase increases its catalytic activity and may thereby regulate the rate of catecholamine biosynthesis *in vivo*. Phosphorylation of rhodopsin decreases its ability to activate rod outer segment phosphodiesterase, and may thereby regulate retinal sensitivity to light *in vivo*. DARPP-32 and G-substrate have been shown to inhibit protein phosphatase activity *in vitro* and phosphorylation of these proteins increases their potency as phosphatase inhibitors. It is possible that phosphorylation of DARPP-32 and G-substrate, through inhibition of specific protein phosphatases, leads indirectly to a physiological response by regulating the state of phosphorylation of other neuronal substrate proteins. As an illustration of the discovery and characterization of a new neuronal phosphoprotein, it is worth considering synapsin I in detail.

Synapsin I

Previously termed protein I, synapsin I was first identified as a phosphoprotein in particulate synaptic fractions of brain³¹. It has been purified to homogeneity from bovine³² and rat³³ brain and extensively characterized. Some of its biochemical, anatomical and physiological properties are summarized in Table 2.

Synapsin I is an effective substrate protein for at least three distinct protein kinases in brain and undergoes multisite phosphorylation. One serine residue (site 1) of the molecule is phosphorylated both by cyclic AMP-dependent protein kinase and by calcium/calmodulin-dependent protein kinase I^{4,7,33}. Two other serine residues (sites 2 and 3) are phosphorylated by calcium/calmodulin-dependent protein kinase II^{4-6,33}. For each of these three protein kinases, synapsin I is one of the most effective substrate proteins known (see ref. 1).

Synapsin I, present only in neurones, appears to be associated with almost all synaptic vesicles in all nerve terminals^{32,34-40}, and represents ~6% of the total protein present in highly purified preparations of synaptic vesicles⁴⁰. It is an extrinsic protein of synaptic vesicle membranes, located on their outer or cytoplasmic surface^{39,40}. Synaptic vesicles appear to contain a specific, saturable and high-affinity ($K_d = 3$ nM at 40 mM salt) binding site for synapsin I⁴¹. Recent studies have suggested that the domain of synapsin I containing phosphorylation sites 2 and 3 is the region of the molecule that binds to synaptic vesicles, and that phosphorylation of sites 2 and 3 decreases this binding (refs 40, 41, and J.-P. Doucet, W. Schiebler, R. Jahn, J. E. Rothlein and P. G., unpublished observations). It is possible that phosphorylation of site 1 of synapsin I affects its interaction with some other component of the nerve terminal, such as the cytoskeleton or plasma membrane.

The state of phosphorylation of synapsin I has been shown to be specifically regulated by serotonin^{42,43}, dopamine⁴⁴⁻⁴⁶ and noradrenaline (P. L. Mobley and P. G., in preparation) in a number of well-defined, relatively homogeneous regions of the central and peripheral nervous systems. In each of these regions, the respective neurotransmitter stimulates the phosphorylation of synapsin I in presynaptic nerve terminals (see ref. 1) apparently via an increase in cyclic AMP levels. These studies have revealed the existence of some previously undetected presynaptic neurotransmitter receptors⁴²⁻⁴⁶ and confirmed the existence of others (S. I. Walaas and P. G., in preparation). In most of the regions examined, it was found that the same type of neurotransmitter receptor is present on presynaptic nerve terminals and on postsynaptic cell bodies and dendrites, suggesting that a dual action of a neurotransmitter at a single synapse is a common synaptic mechanism. According to this idea, a given neurotransmitter acts both on presynaptic nerve terminals, where it regulates the amount of a second neurotransmitter released in response to nerve impulses, and on postsynaptic cell bodies and dendrites, where it regulates the responsiveness of the postsynaptic element to that second neurotransmitter. Thus, study of synapsin I phosphorylation has provided a unique method for detecting and characterizing presynaptic receptors directly, as well as for determining their role in synaptic transmission (see ref. 1).

Brief periods of nerve impulse conduction, at physiological frequencies, increase the phosphorylation of synapsin I in nerve terminals of the rabbit superior cervical ganglion^{45,47}, via an influx of calcium into the nerve terminals. Preganglionic nerve stimulation results in the conversion of about 80% of presynaptic synapsin I from the dephosphorylated to the phosphorylated form⁴⁵ at the rate of about 2% of the total presynaptic synapsin I per preganglionic nerve impulse (calculated from data in refs 45 and 47). Nerve impulse conduction has also been shown to increase the phosphorylation of synapsin I in nerve terminals of the posterior pituitary⁴⁶.

The localization of synapsin I to the cytoplasmic surface of synaptic vesicles, and the regulation of its state of phosphorylation by first messengers that increase neuronal calcium or cyclic AMP levels, have led us to hypothesize that it has a role in regulating the process by which neurotransmitter is released from the nerve terminal. As, in addition, it has been much easier to demonstrate cyclic AMP-dependent or calcium-dependent regulation of neurotransmitter release from nerve terminals (which always contain synapsin I) than from chromaffin cells which lack it^{34,37}, we consider it more likely that synapsin I plays a part in a neurone-specific regulation of the release process, than in the release process itself.

What role might synapsin I have in the regulation of neurotransmitter release? An elevation in either calcium or cyclic AMP has been shown to potentiate the release of neurotransmitters from various nerve terminals in various experimental conditions. For example, brief periods of impulse conduction, apparently acting through calcium, increase the amount of neurotransmitter released in response to a single nerve impulse in numerous types of neuronal preparations, a process known as 'post-tetanic potentiation'^{48,49}. Similarly, cyclic AMP, and those neurotransmitters that act through cyclic AMP, increase the amount of neurotransmitter released, in response to a single nerve impulse, from several types of nerve terminals (see ref. 50 for review), including those at the neuromuscular junction⁵¹, at invertebrate axo-axonic synapses¹⁰, in sympathetic ganglia⁵² and in the central nervous system^{53,54}. As discussed above, brief periods of impulse conduction, apparently acting through calcium, and neurotransmitters, apparently acting through cyclic AMP, also stimulate the phosphorylation of synapsin I in a variety of nerve terminals. Moreover, cyclic AMP-dependent protein kinase and calcium/calmodulin-dependent protein kinase I phosphorylate synapsin I on the same individual serine residue (in site 1). It is possible, therefore, that an increase in the state of phosphorylation of synapsin I at site 1 represents a common molecular pathway through which brief periods of impulse conduction and certain neurotransmitters facilitate neurotransmitter release at the synapse.

Conclusion

Twenty years ago, there was almost no information available concerning the molecular machinery by which chemical and electrical stimuli produce diverse physiological responses in various types of neurones. In fact, there was not even a conceptual framework available by which one could approach this subject. As a result of work over the past 15 years, it has become apparent that the study of protein phosphorylation pathways provides such an approach. Studies involving the injection of protein kinases or protein kinase inhibitors into neurones and other excitable cells have provided direct evidence that activation of protein kinases is an obligatory step in the sequence of events by which extracellular signals produce certain physiological responses in these cells.

The large number of cell type-specific neuronal phosphoproteins already found provides strong evidence that there is a high degree of chemical specificity among neurones, in support of the notion that it should ultimately be possible to develop numerous classes of therapeutic agents that are selectively targeted at specific neurone populations. Such agents

would offer new approaches to the treatment of neurological and psychiatric disorders.

The protein kinase/protein phosphatase system, by controlling the state of phosphorylation of substrate proteins in every conceivable category, provides the type of flexibility required for the regulation of a variety of neuronal processes, including

processes having widely different temporal characteristics. By studying protein phosphorylation mechanisms, it should be possible to build a progressively more complete understanding of the molecular basis of innumerable types of neurophysiological phenomena, from the shortest-lived to the longest-lived, and from the most simple to the most complex.

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Frequencies, amplitudes and linewidths of solar oscillations from total irradiance observations

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Ten months of solar total irradiance data from the Solar Maximum Mission satellite have generated accurate frequencies, amplitudes and linewidths for individual ~ 5 -min solar p-mode oscillations of low degree. The modes can be described as independent and chaotically excited oscillators, and provide no evidence for the fine structure taken to imply rapid internal rotation of the Sun.

SOLAR seismology, the study of the Sun's interior structure and dynamics through observations of its normal-mode oscillations, is developing rapidly, both observationally and theoretically. The ~ 5 -min p-modes of low-degree (l) which penetrate the solar core, were first observed in line-of-sight velocity (Doppler) measurements of the solar surface, made in integral sunlight¹. Velocity observations of low-, intermediate-, and high- l p-modes have generally confirmed standard assumptions about solar structure, but significant discrepancies remain². Resolution of these discrepancies may eventually illuminate the solar neutrino problem.

The Birmingham group³ has found a multiplet structure in the velocity power spectrum of the low- l oscillations. Slow, uniform rotation will split the nominally degenerate m-states of fixed n and l into $2l+1$ equally spaced frequency components⁴. The magnitude of the observed splitting suggests rapid rotation of the solar interior, perhaps in the presence of a strong internal magnetic field^{3,5,6}. The precise interpretation of the 'fine structure' is still controversial⁷.

Aside from its interpretation, the detection of such fine structure requires the individual members of a multiplet to have suitably small frequency widths, or the members will blend together in the spectrum. In fact, Claverie *et al.*³ quote a full width for both the largest-amplitude singlet ($l=0$) modes, and the individual members of the triplet ($l=1$) modes, as $0.5 \mu\text{Hz}$, not appreciably larger than the resolution of their 28-day data set.

The Nice group, however, claims⁸ that at least some of the modes, those with frequencies above 3 mHz, are intrinsically too broad to display splitting. The Nice power spectrum was incapable of showing splitting because of the short time span of the observations (5 days). The Nice group also claimed to observe large amplitude excursions in all the low- l p-modes over periods as short as 12 hours. They concede that modes below 3 mHz, which they could not resolve, might be narrow enough to show splitting, provided the amplitude swings they saw were caused by amplitude modulation only. This leaves open the question of the phase coherence time.

We have studied the low-degree ($l=0, 1$, and 2) oscillations with the total solar flux data from the Active Cavity Radiometer Irradiance Monitor (ACRIM) experiment on board the Solar Maximum Mission spacecraft (SMM)^{9,10}. In our early reports^{11,12}, we described the spectral analysis of the first ~ 5 months of data, confirming the frequencies of the Birmingham and Nice groups and establishing the mean irradiance amplitudes of the brightest modes, but leaving the question of precise linewidths and fine structure open. This article describes a more complete analysis, based on the entire ~ 10 months of usable data from the middle of February 1980 until the loss of space-

craft fine-pointing control in December. With the additional data, the frequencies, amplitudes and linewidths of individual modes have been measured more accurately. A Fourier analysis with the frequency resolution of the entire data set has failed to confirm either the fine structure, or the narrow linewidths this structure implies.

Irradiance observations

The sensor of the ACRIM experiment is a specular-finish black cone which absorbs solar radiation with near-perfect efficiency over most of the spectrum. The cone is at all times heated resistively and loses energy to a thermal sink. A mechanical shutter blocks the solar input during half of a 131.072 s cycle. The total heating rate is kept fixed by servo-adjustment of the electrical input, hence the perceived solar flux is proportional to the difference of the shutter-open and shutter-closed ohmic heating rates. The voltage across the heater coil is digitized and telemetered every 1.024 s. The first 32 readings in both the open and closed periods are not used because of the transient servo response following the closing or opening of the shutter. For each shutter cycle both a closed and an open ohmic heating rate are computed by averaging the final 32 readings from the corresponding half of the cycle. We estimate the solar flux at the midpoint of the usable part of a given shutter-open period taking the difference of the open ohmic heating rate and the average of the two shutter-closed measurements immediately preceding and following the given open period. The thermal response time of the instrument is only a few seconds⁹, and the sensitivity is limited by instrumental noise of ~ 30 parts per million (of the solar flux) r.m.s. fluctuation over a shutter open period.

In our analysis we work with the series of shutter cycle averages in which variations caused by the changing Earth-Sun distance and by the orbital motion of the satellite have been compensated (the orbital effect includes a first-order relativistic transformation of the Poynting flux). The resulting series corresponds to the flux variation apparent to an observer in a circular orbit about the Sun. The residuals, after removing the mean, are expressed in units of the mean solar irradiance. This series of measurements contains regular gaps corresponding to the night portion of the ~ 96 minute SMM orbit. The relative power in the n th orbital harmonic of the window function is very close to $\sin^2(n\pi a)/(n\pi a)^2$, with $a = 3/5$, and thus consistent with a square-wave observing window with $3/5$ data coverage. The window function spectrum contains, in addition, a small noise contribution caused by other assorted gaps. The overall data coverage, taking all gaps into account, was $\sim 43\%$, in a series of measurements spanning ~ 290 days.

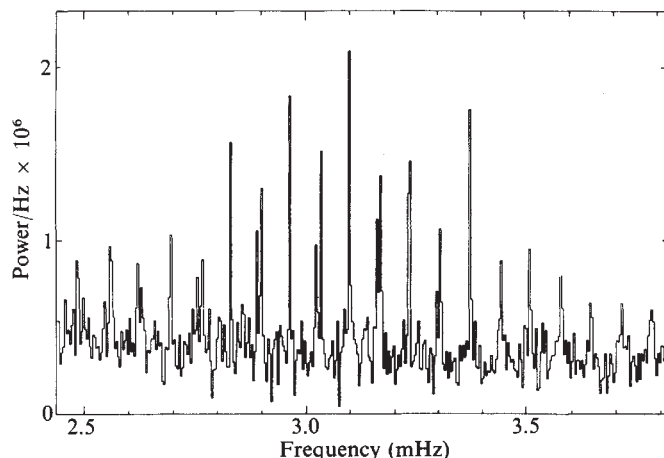


Fig. 1 Power spectrum of solar total irradiance variations from the ACRIM instrument on board the Solar Maximum Mission spacecraft, showing the ~ 5 -min p-modes of degree $l=0, 1$ and 2 . This spectrum was computed from an unbiased estimate of the auto-correlation function of the time series, to suppress sidebands induced by data gaps. The frequency resolution of the plot is $3.725 \mu\text{Hz}$, enough to separate peaks belonging to different (n, l) values. The principal peaks in the plot correspond to sine-wave amplitudes of 2 – 4 parts per million of the mean irradiance. The heights of the peaks define an envelope of power versus frequency, centered at 3.1 mHz having a full width of approximately 0.7 mHz.

Analysis

The simplest way of estimating the power spectrum of flux variations is to calculate the discrete Fourier transform of the series. Due to occasional random resetttings of the SMM master clock, the sampling in time is not absolutely uniform. For this reason we truncated the actual observation times to a 32.768 s grid to allow computation with a fast Fourier transform (FFT) algorithm. Grid points for which actual data are unavailable (for example, during data gaps) default to the value zero.

In a spectrum estimated by the FFT method an oscillatory spike is accompanied by a set of aliases, or sidebands, caused by the orbital gaps. An alternate method of deriving the spectrum, which tends to suppress the aliases, is to Fourier-transform an unbiased (except for a taper) estimate of the auto-correlation function (ACF). One drawback of the ACF approach in analysing ACRIM data is caused by the SMM clock resets, which create irregular sampling in the time-lag domain on a time grid finer than the shutter cycle duration. Therefore, we are forced to use the coarser 131.072 s binning when computing an ACF. The ACF method gives an unbiased estimate of the mean square irradiance variations of both the oscillations and of the continuum variations, while the straight FFT procedure is preferred for high frequency resolution. Finally, to suppress large low-frequency trends in the data, the above methods of analysis have been applied only to the series of differences of consecutive shutter-cycle averages.

Results

Figure 1 shows a power spectrum, obtained by the ACF method, of the low- l p-modes. The sine-wave amplitudes of the brightest modes, a few parts per million of the mean irradiance, agree with rough theoretical estimates based upon velocity amplitudes^{12–14}. All of the results to be described below were obtained from a periodogram (spectrum) of ~ 290 days of data.

Frequencies: Each of the frequencies listed in Table 1 is the centroid of the power distribution (excess over continuum) within a $5 \mu\text{Hz}$ interval centred on an initial frequency estimate. The $l=0$ and $l=2$ mode sequences, which nearly overlap, blend together above 3.4 mHz in our spectrum, probably because of

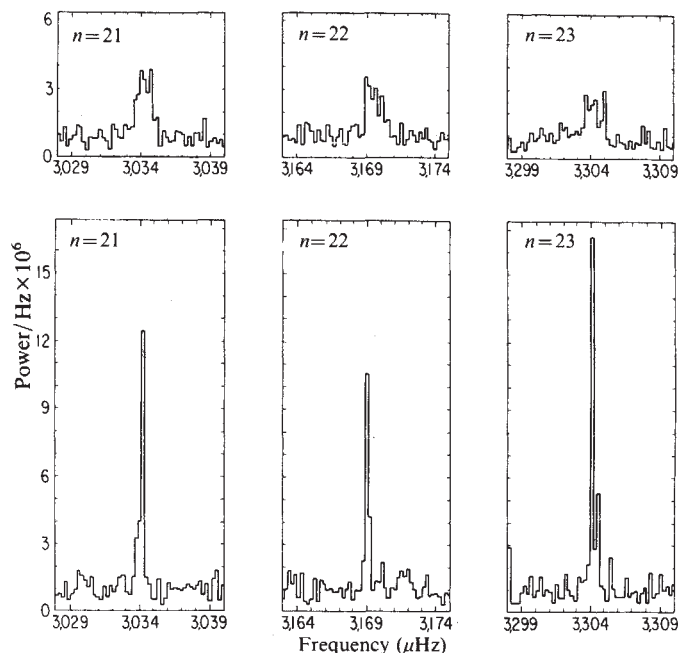


Fig. 2 Top panels, examples of singlet ($l=0$) modes at a resolution of $0.2328 \mu\text{Hz}$, from a spectrum computed by direct FFT of ~ 290 days of ACRIM data. The widths of these modes, as estimated by the method described in the text, are $>1.0 \mu\text{Hz}$. Bottom panels, artificial singlet modes, shown for comparison, were generated to test the validity of the entire analysis procedure (see text). The intended line widths ($0.3 \mu\text{Hz}$), frequencies, and amplitudes (set approximately equal to the estimates for the corresponding real modes) of the synthetic oscillations are faithfully reproduced by analysis, within statistical uncertainty.

increasing line width. Below 2.5 mHz the modes decrease in amplitude while the background noise increases.

We have fitted each sequence of frequencies belonging to a given l to a relation of the form

$$\nu_{nl} = a_l(n-21)^2 + b_l(n-21) + c_l \quad (1)$$

for the cases $l=0, 1$, and 2 , using the modes listed in Table 1. The results are presented in Table 2. The scatter of the points about the best-fit curves is roughly twice the formal errors, suggesting true deviations from a smooth fit.

Amplitudes: We have computed the mean square irradiance fluctuation for a given mode by taking the power above background over a $5 \mu\text{Hz}$ band centred on the frequency in Table 1. The results are listed in Table 3.

Linewidths: We have estimated the linewidths of the dominant $l=0$ modes, which should not be split by rotation or other time-independent perturbations. Examples of these are displayed in the top panels of Fig. 2. These modes appear significantly broader than the $0.5 \mu\text{Hz}$ upper limit on the full width claimed by Claverie *et al.*³. To quantify this we compute, for each of the singlet modes $n=19$ to $n=24$, the width of the centred frequency band containing half the total power of the mode, and tabulate the results in the second column of Table 3. For a lorentzian line profile this definition is the same as the full width at half maximum.

The appearance of the main $l=1$ modes in our spectrum is similar to that of the singlets; they show no obvious triplet structure. Our data are considerably noisier than the velocity data, however. To improve the statistics we have superposed the main $l=1$ peaks (see Fig. 3) on the assumption that this sequence of modes follows the trajectory defined by equation 1 and Table 2. This superposition method yielded triplets in the Birmingham spectrum (see Fig. 2a of ref. 3). Although the composite profile we obtain for the $l=1$ modes does not have

Table 1 Frequency centroids (μHz)

Degree l	0	1	2
Order n			
17		$2,559.20 \pm 0.20$	$2,619.65 \pm 0.38$
18	$2,629.46 \pm 0.34$	$2,693.68 \pm 0.18$	$2,754.96 \pm 0.34$
19	$2,765.03 \pm 0.26$	$2,828.73 \pm 0.14$	$2,890.04 \pm 0.24$
20	$2,899.14 \pm 0.15$	$2,963.50 \pm 0.13$	$3,024.54 \pm 0.17$
21	$3,034.02 \pm 0.12$	$3,098.66 \pm 0.10$	$3,159.99 \pm 0.16$
22	$3,169.40 \pm 0.12$	$3,233.43 \pm 0.11$	$3,295.23 \pm 0.26$
23	$3,303.77 \pm 0.15$	$3,369.50 \pm 0.12$	$3,433.17 \pm 0.45$
24	$3,439.81 \pm 0.20$	$3,505.05 \pm 0.19$	
25		$3,642.47 \pm 0.30$	

Table 2 Coefficients to best-fit polynomials (μHz)

	$l=0$	1	2
a	0.06 ± 0.02	0.12 ± 0.01	0.16 ± 0.04
b	134.94 ± 0.05	135.25 ± 0.03	135.59 ± 0.10
c	$3,034.11 \pm 0.08$	$3,098.48 \pm 0.06$	$3,160.05 \pm 0.11$

Table 3 Power (p.p.m.²) and linewidths (μHz)

n	$l=0$		$l=1$	$l=2$
	Power	Width	Power	Power
17			2.98 ± 0.68	1.86 ± 0.57
18	1.71 ± 0.53	1.7	3.34 ± 0.71	2.00 ± 0.56
19	2.09 ± 0.56	1.1	4.54 ± 0.83	2.24 ± 0.59
20	4.30 ± 0.75	1.3	5.74 ± 0.86	3.33 ± 0.67
21	4.75 ± 0.84	0.9	7.63 ± 1.00	3.75 ± 0.68
22	4.44 ± 0.80	0.9	6.96 ± 0.93	1.97 ± 0.51
23	3.94 ± 0.69	1.3	5.89 ± 0.83	1.14 ± 0.42
24	2.51 ± 0.54	1.5	2.94 ± 0.57	
25			1.89 ± 0.46	

a triplet appearance, it is definitely broader than the profile of the singlets.

Sources of error: The errors in Tables 1–3 were computed assuming that the coefficients in the basic Fourier transform of the series are independent gaussian random variables, and that the line profiles of the multiplet substates are lorentzian. Some motivation for these assumptions is provided by the theory of excitation and damping by turbulent convection¹⁵.

The effects of aliasing due to the on-off modulation by both the satellite orbit and the shutter action were considered, although they were ignored in computing formal errors. Only the first-order orbital sidelobes of true modes in the spectrum are of appreciable amplitude, and they fortunately do not overlap any other true modes. The Nyquist frequency ν_{NY} of the shutter cycle is 3.8147 mHz. A mode at frequency $\nu > \nu_{\text{NY}}$ will produce a ghost at $\nu' = 2\nu_{\text{NY}} - \nu < \nu_{\text{NY}}$. The ghosts of the odd (even) l modes happen to lie close to the even (odd) l modes. This circumstance complicates the analysis of modes slightly below ν_{NY} . The effect on modes below 3.4 mHz is estimated to be unimportant in view of the rapid decrease in the height of the peaks (hence their aliases) in the spectrum with frequency (see Fig. 1). As other observers have noted^{18,16}, the linewidths increase rapidly with frequency, which probably explains most of the dropoff (our spectrum is consistent with this interpretation, but shutter aliasing contributes to the apparent increase). We are more concerned with modes below 3.4 mHz, since the Birmingham interpretation requires that the dominant modes, at least, be split. For these modes interference from aliases does not seriously affect our measurements.

To check for unexpected sources of line broadening (or other distortion) arising purely from our method of data handling, we have performed the entire analysis on a synthetically generated time series. The series simulated both frequency-stable oscillations, including fine structure as observed by Claverie *et al.*³,

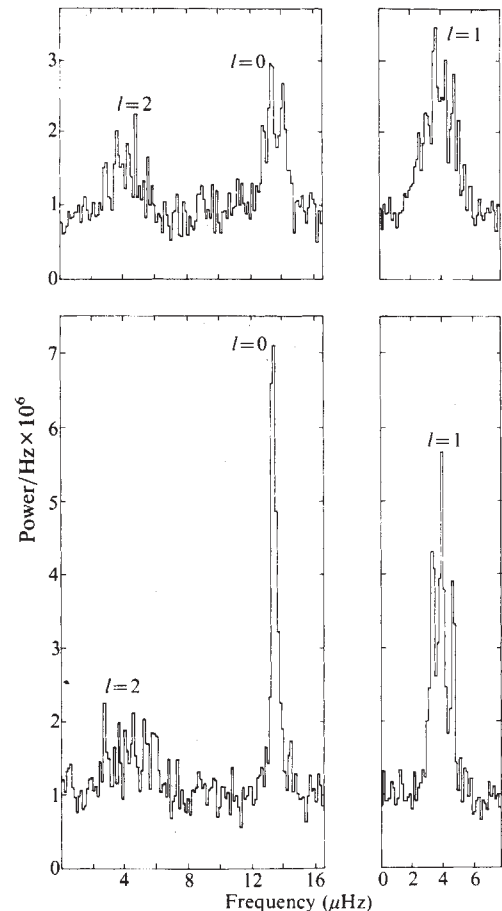


Fig. 3 Top panels, superposed frequency analysis of real modes from Table 1, based on the best-fit frequency polynomials (equation 1, Table 2). The inclusion of the quadratic correction in the summing technique was suggested by E. Fossat. Left, composites of both $l=0$ and 2 modes, based on the $l=0$ best-fit polynomial. Right, composite of the $l=1$ modes, using the $l=1$ best-fit polynomial. The composite of the triplet modes is manifestly broader than the mean profile of the singlets, but this enhancement is consistent with either the original rapid-rotation interpretation of Claverie *et al.* or with rigid rotation of the Sun at the observed surface rate. Bottom panels, same analysis, performed on the spectrum of synthetic modes, demonstrates that narrow triplets should be visible in the superposed spectrum of ACRIM data provided the frequencies are stable over a 10 month interval. (Note: slight distortions of a true frequency scale cannot be avoided because of the quadratic term in the fitting polynomials.)

and 'continuum' variations. The continuum variations were modelled after the overall spectrum of irradiance data, while the substates of the various multiplets were each modelled after a damped harmonic oscillator driven by a broad-band noise source. The total power, shared equally between components, and approximate frequency centroid of each synthetic multiplet were taken from the spectrum of actual ACRIM data. The widths of the individual triplet components were set at 0.3 μHz (lorentzian profile), and their separations at 0.67 μHz , slightly less than the Birmingham result. The response of the instrument to these artificial flux variations was realistically modelled and the analysis of the resulting voltage data was identical to the analysis of the original ACRIM data, including masking of the time series by the observing window of the real data.

Beyond these sources, the only non-solar causes of line-broadening which would seriously alter our conclusions are errors in the reported times of observation or errors in the measurement of flux itself. Time inaccuracies effectively broaden a purely harmonic signal (phase modulation) only when the error is a significant fraction of the signal period. We have assumed that the SMM time information provided by the

National Aeronautics and Space Administration satisfies our requirements, and note that B. Dennis (private communication) has confirmed the accuracy of the times independently from a comparison of solar flare times. The peaks in the spectrum would not be artificially broadened by errors in measured flux unless the ACRIM sensitivity to short-period oscillations were to vary systematically with time.

The bottom panels of Fig. 2 are the $l=0$ modes from the spectrum of the synthetic time series. Comparison with the corresponding real modes shows no evidence of artificial broadening. Comparison of real and artificial triplet modes (Fig. 3) demonstrates that our spectral analysis is capable of resolving the triplet structure. The frequencies and amplitudes of all the synthetic modes are also undistorted by the analysis.

Discussion

Systematic frequency differences occur among the three main 1980 data sets. The frequencies of the Birmingham group³ tend to be smaller by $\sim 1 \mu\text{Hz}$ than ours, while those of the Nice group⁸ average higher by about the same amount. We have also fitted the polynomials defined by equation 1 to the velocity data and to theoretical eigenfrequencies, using only modes in Table 1. Both the Nice and SMM data show positive 'curvature' coefficients a_b , while the coefficients obtained from the Birmingham data are consistent with zero. The mean frequency spacing b_l increases monotonically with l in our data, but changes erratically in the other data sets. The origin of these discrepancies is unknown, but could be related to the 'line-pulling' effects mentioned by Claverie *et al.*³. The centroids of all three data sets occurred within a 7-month span. The l -dependence of the coefficients in Table 2 compares more favorably with the values computed from lists of theoretical frequencies^{2,17,18}, than with other observations. In view of the simulation described above we regard the frequencies obtained from an irradiance measurement to be trustworthy, at least for the limited selection of modes presented here, with large signal-to-noise ratio.

These more accurate frequency measurements will be useful in further constraining models of the solar interior. Since the discrepancies between existing solar models and observations are at present much greater than among the observations themselves², the main advantage of better measurements lies in the possibility of detecting long-term (such as solar cycle) frequency variations.

The unexpectedly broad lines and the absence of fine structure in our spectrum could be explained by slow drifts in the mode frequencies by $\sim 1 \mu\text{Hz}$ over the 10-month ACRIM observing interval. The blurring of fine structure by such gradual changes need not be apparent in a single month of data. Gough has pointed out that changes of this magnitude might be expected in connection with the solar cycle¹⁹. The observation of triplets by the summed-epoch technique (refer to Fig. 2a. of ref. 3) can be reconciled with frequency changes only if the modes drift in unison. Drifts of this kind would occur if the run of sound speed in the solar interior were to vary.

From the linewidths of the ACRIM spectrum alone, a lower limit can be placed on the phase coherence time of the modes. For a lorentzian line shape, the full width at half maximum $\Delta\nu$ is related to the e -folding time for the decay of modal power τ by

$$\Delta\nu = 1/2\pi\tau \quad (2)$$

which, taken as a definition of phase coherence time, gives $\tau_{\text{phase}} \approx 2$ days for the main $l=0$ modes. Even this strong lower limit on the phase lifetime of these modes is about a factor of 10 greater than a recent theoretical prediction²⁰, indicating the need for further investigation of wave dissipation mechanisms. (Note that in our initial report¹¹ we implicitly defined the coherence time without the 2π .) The more stringent condition, $\Delta\nu < 0.3 \mu\text{Hz}$, necessary for the observation of fine structure, corresponds to $\tau_{\text{phase}} > 6$ days.

Each of the three sequences of modes, $l=0, 1$, and 2 , seems to define a bell-shaped envelope of power versus frequency.

The envelopes each peak near 3.1 mHz and are roughly 0.7 mHz wide, but differ in height (see Fig. 1 or Table 3). This observation suggests an indirect way of estimating the amplitude lifetimes of the modes, provided they are driven independently. For a chaotically driven harmonic oscillator, the power estimated from a data string of duration T , deviates from its infinite-time average value. The mean square fractional deviation σ^2 is given by

$$\sigma^2 = 2\tau/T \quad (3)$$

provided $T \gg \tau$, or equivalently, $\sigma^2 \ll 1$. Equation 3 is our definition of amplitude lifetime, τ_{amp} . The infinite-time average power of each of the singlet modes $n=19, 24$ was estimated by fitting a parabola to these points. The dispersion of modal power about this parabola, then, gives an upper limit on τ_{amp} , since for independently excited oscillators, an ensemble average (6 modes, 3 degrees of freedom) approximates a time average. The dispersion in the spectrum is expected to be larger than the value given by equation 3 because of noise interference. Assuming the Fourier amplitudes of the data (signal plus noise) to be normally distributed, we find a formal probability for this collection of modes to reproduce the observed dispersion (or less) of 0.05, if τ_{amp} is 6 days or more. Because the true envelope is probably not a parabola, this upper limit is even stronger.

Since τ_{amp} is unlikely to be as much as 6 days, conflict with the Birmingham interpretation can only be avoided if significantly more amplitude modulation occurs than the simple harmonic oscillator model would imply, or if the mode amplitudes are correlated. The possibility of pure amplitude modulation has been raised by the Nice group⁸, though a physical mechanism seems to be lacking. Although the 12-h (or shorter) amplitude lifetime claimed by this group is consistent with our upper limit, the possibility that the apparent modal power variations in 1/2-day intervals come from the background interference (albeit small) in their data cannot be dismissed.

The explanations given above, which might reconcile our findings with those of the Birmingham group (frequency drifts and amplitude modulation), are *ad hoc* and not easy to verify in our noisy data. The lower limit on the coherence lifetime, $\tau_{\text{phase}} \approx 2$ days, and the upper limit $\tau_{\text{amp}} < 6$ days, based just on ACRIM data are to be expected if all the line broadening is the result of natural damping (if $\tau_{\text{amp}} = \tau_{\text{phase}}$). This much more reasonable hypothesis eliminates the need for both frequency drifts and pure amplitude modulation mechanisms, provided some alternative interpretation of the fine structure is available. That the fine structure is an artifact of the analysis has already been suggested⁸. We have performed a superposed-frequency analysis on a spectrum of broad-band noise and find seeming fine structure to result from oversampling in frequency. If the modes are described by broad resonance envelopes and are excited chaotically, as our data suggest, they would most likely show spurious fine structure, even when superposed.

While the detailed features (multiplet components) in the Birmingham spectrum are probably not significant, the overall widths of the superposed $l=1$ and $l=2$ modes do place limits on the amount of splitting. The magnitude of the splitting quoted by Claverie *et al.*³ should be regarded as an upper limit. The composite line width of the main $l=1$ peaks (see Fig. 3) in our spectrum does not allow us to distinguish this upper limit from the splitting caused by uniform rotation. Because the composite profile of the triplets is visibly broader than that of the singlets, the mean interior rotation rate cannot be much less than the surface rate, as Claverie *et al.* have already noted. (We assume that the triplet substates have the same intrinsic widths as the singlet states.) To infer the exact core rotation rate from high-order (n) p-mode observations, a moderate degree of spatial resolution will probably be necessary, to separate the m-states. Rotational splitting in lower- n p-modes, having presumably longer coherence times, may be resolvable with full-disk measurements. These latter modes cannot be separated from the noise in our spectrum. Low- l g-modes may also provide information about internal rotation²¹.

Conclusions

The most straightforward interpretation of the power spectrum of total irradiance data is that the low- l p-modes behave like independent, randomly excited harmonic oscillators. The damping time $\tau \approx 2$ days of the multiplet substates, inferred directly from the line widths of the main $l=0$ modes and indirectly from the scatter in the power of the modes, is incompatible with the previous observation of rotationally split components. The composite width of the unresolved $l=1$ peaks in our spectrum is

consistent with either the amount of splitting ($0.75 \mu\text{Hz}$ per m-state) claimed by Claverie *et al.*, which we take as an upper bound, or with the splitting expected from uniform rotation at a rate of $\sim 0.43 \mu\text{Hz}$. We conclude that the precise rotation rate of the solar interior is still an open question.

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Breaking planetary waves in the stratosphere

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Satellite-borne IR radiometers are turning the Earth's stratosphere into one of the best available outdoor laboratories for observing the large-scale dynamics of a rotating, heterogeneous fluid under gravity. New insight is being gained not only into stratospheric dynamics as such, with its implications for pollutant behaviour and the ozone layer, but also indirectly into the dynamics of the troposphere, with its implications for weather forecasting. Similar dynamical regimes occur in the oceans and in stellar interiors. A key development has been the construction of coarse-grain maps of the large-scale distribution of potential vorticity in the middle stratosphere. Potential vorticity is a conserved quantity which has a central role in the dynamical theory, but is difficult to calculate accurately from observational data. We present the first mid-stratospheric potential vorticity maps which appear good enough to make visible the 'breaking' of planetary or Rossby waves, a phenomenon ubiquitous in nature and arguably one of the most important dynamical processes affecting the stratosphere as a whole.

THE flow in the northern wintertime stratosphere is often thought of as consisting of an anticlockwise vortex centred on the cold polar cap, disturbed by a pattern of 'planetary waves'. Two examples are shown in Fig. 1a, b, which are conventional maps showing the height of the 10-mbar isobaric surface on 17 and 27 January 1979. This surface lies in the middle stratosphere, at heights ranging between about 28 and 32 km. Because there is approximate geostrophic balance between horizontal pressure gradient and Coriolis acceleration, the air flow is nearly parallel to the height contours, as suggested by heavy arrows in Fig. 1. Velocities in the fastest parts of the stream may reach about 75 m s^{-1} ($\sim 90^\circ$ longitude per day at 50°N). The pattern in Fig. 1a contains a wave-3 disturbance, consisting of three pairs of ridges and troughs around a latitude circle, while Fig. 1b is dominated by a wave-1 disturbance, with a single trough over Europe. This wave-1 disturbance was observed to be nearly stationary with respect to the Earth's surface, and had an unusually large amplitude. It was the first of a sequence of events leading several weeks later to a major sudden warming, during which the circumpolar vortex broke up completely and temperatures in the polar cap rose by tens of degrees in just a few days.

The most prominent wave patterns at these altitudes in winter tend to be more or less stationary and to be dominated by the largest spatial scales, associated with waves 1 and 2 and to a lesser extent with wave 3. As is well known, this fact is in

qualitative agreement with the predictions of linear wave theory, if one assumes that the primary source of the waves is in the much denser troposphere below and involves processes tied to large-scale geographical features, favouring the generation of stationary waves. Linear theory predicts that those stationary waves which have the largest horizontal scales penetrate highest.

The dynamical restoring mechanism, whereby the waves can propagate westward relative to the stream and remain stationary on the polar vortex, depends on the existence of a cross-stream gradient of Ertel's potential vorticity in isentropic surfaces. Part of this cross-stream gradient is due to the varying direction of gravity relative to the Earth's rotation axis, the so-called planetary vorticity gradient or 'beta effect', and there are further important contributions dependent on the velocity profile of the airstream. Ertel's potential vorticity Q is defined by

$$Q = \rho^{-1} (2\boldsymbol{\Omega} + \nabla \times \mathbf{u}) \cdot \nabla \theta \quad (1)$$

where $\boldsymbol{\Omega}$ is the Earth's angular velocity, $\mathbf{u}$ is air velocity relative to the Earth, ρ is air density and θ specific entropy, the gradient of which is nearly vertical in the stratosphere because the isentropic surfaces are nearly horizontal. One may equally well take θ to be potential temperature, or any other function of specific entropy. The equations of motion imply that, if the motion is adiabatic, that is, if

$$D\theta/Dt = 0 \quad (2)$$

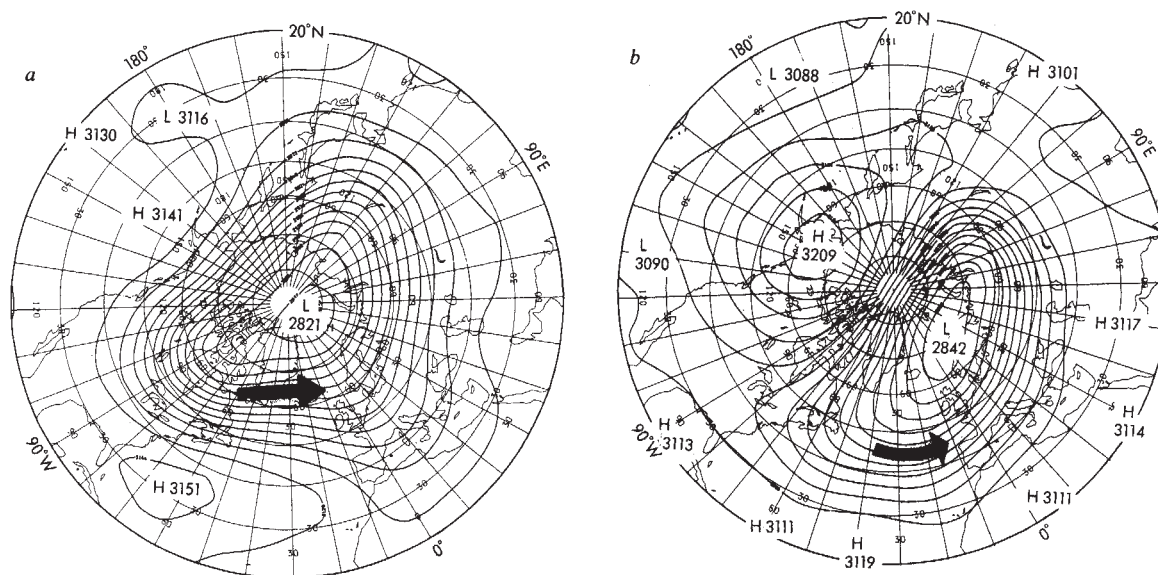


Fig. 1 Geopotential height in decametres (numerically nearly the geometric altitude above sea level) of the 10-mbar isobaric surface on 17 (a) and 27 (b) January 1979, at 00 h GMT. Contour interval is 24 decametres. Map projection is polar stereographic. The southernmost latitude circle shown is 20° N.

then, provided also that the motion is frictionless,

$$DQ/Dt = 0 \quad (3)$$

(Ertel's theorem). $D/Dt = \partial/\partial t + \mathbf{u} \cdot \nabla$ is the rate of change seen by an individual air parcel; thus, Q and θ are constant following the motion. For present purposes this is a qualitatively reasonable approximation, for time scales up to a week or so. Over longer time scales, and in any case for quantitative work, the effects of radiative heating or cooling would have to be included, especially at the highest levels in the stratosphere. Equations (2) and (3) imply that when material contours are displaced northward and southward in a wavy pattern, the contours of potential vorticity in an isentropic surface are similarly displaced. The velocity field associated with the resulting potential vorticity disturbance lags the displacement pattern by a quarter wavelength, implying that the whole pattern propagates westward relative to the stream. This wave propagation mechanism was identified in 1939 by C.-G. Rossby, who recognized its importance for large-scale flow in the troposphere. It is an essentially linear mechanism. Of course, equation (3) itself applies equally well to nonlinear as to linear phenomena. A time sequence of isentropic maps of Q provides not only a good way of seeing when and where the Rossby-wave propagation mechanism is effective (and hence of judging the possible relevance of associated theoretical concepts like group velocity, refractive index, and so on), but also provides a very direct insight into some of the more important consequences of non-linearity.

Data and approximations

Contour maps of Q on the 850 K isentropic surface in the Northern Hemisphere, at daily intervals during January–February 1979, were constructed at the UK Meteorological Office. 850 K is the potential temperature defined as the temperature of an air parcel brought adiabatically to a nominal sea-level pressure of 1,000 mbar. The data used were 50- and 100-mbar height fields from conventional meteorological objective analyses (NMC, Washington), together with IR spectroscopic radiances from channels 25 and 26 of the prototype Stratospheric Sounding Unit on board the polar orbiting satellite Tiros-N. Channels 25 and 26 receive radiation mainly from the region above 100 mbar, and their weighting functions peak at

about 16 and 6 mbar, respectively¹. The 850 K isentropic surface was chosen because it lies mainly between the peaks of the weighting functions and is one of the most suitable isentropic surfaces, in terms of signal-to-noise ratio, on which to estimate the derivatives required for calculating Q . It is also close to the 10-mbar isobaric surface depicted in Fig. 1, and is centrally placed for observing stratospheric planetary-wave phenomena.

To estimate Q , smoothed height fields on the 20, 10, 5, 2 and 1 mbar isobaric surfaces were derived from the hydrostatic relation and the IR radiances, using standard techniques summarized in Fig. 3 of ref. 1; this is also how the maps in Fig. 1 were obtained. The 1- and 2-mbar fields were inaccurate through failure of the third IR channel, 27, but we believe that this did not materially affect the analysis near 10 mbar. The so-called gradient wind approximation, which assumes geostrophic balance plus a correction allowing for the local curvature of air parcel trajectories, was used to estimate the vertical component ζ of the vorticity vector, $\nabla \times \mathbf{u}$, on each isobaric surface. A simple centred-difference formula having second-order accuracy was used, the height fields having been interpolated onto a square grid on a polar stereographic projection, the grid size being about 580 km at 50° N. The pressure p on the 850 K isentropic surface was then found at each grid point by a cubic spline interpolation procedure, and ζ was similarly interpolated on to that surface. Q was then found from equation (1), taking θ as potential temperature relative to 1,000 mbar and neglecting the small horizontal component of $\nabla \theta$. In estimating the vertical component $\partial \theta / \partial z$ from the data, the hydrostatic relation $\partial p / \partial z = -\rho g$ was again used, giving

$$p^{-1} \partial \theta / \partial z = -g \partial \theta / \partial p = -g p^{-1} \partial \theta / \partial (\ln p) \quad (4)$$

where z is the altitude and g the acceleration due to gravity; $\partial \theta / \partial (\ln p)$ was estimated as the local slope of the curve defined by the appropriate cubic spline. All the vertical interpolations were carried out with respect to $\ln p$, roughly equivalent to using the true altitude z . Further details are given in ref. 2.

It is emphasized that the satellite data can at best represent smeared-out versions of the temperature and motion fields. The horizontal resolution is limited, especially in the tropics, by the number of orbits, about 14 polar orbits per day. The vertical resolution is limited by the half-widths of the weighting functions, just under 10 km. *A fortiori*, the data cannot resolve the fine-grain structure in the θ and Q fields which is to be expected for various reasons, and evidence of which has frequently been

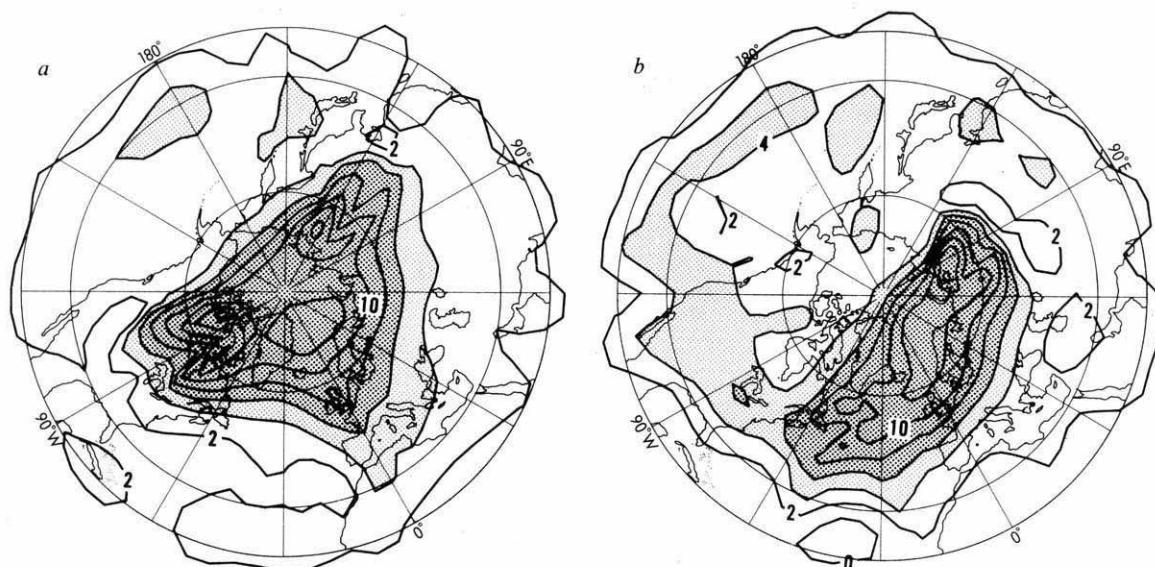


Fig. 2 Coarse-grain estimates of Ertel's potential vorticity Q on the 850 K isentropic surface (near the 10-mbar isobaric surface) on 17 (a) and 27 (b) January 1979, at 00 h GMT. The southernmost latitude circle shown is 20° N; the others are 30° N and 60° N. Map projection is polar stereographic. For units see equation (5) onwards. Contour interval is 2 units. Values greater than 4 units are lightly shaded, and greater than 6 units heavily shaded.

seen in the stratosphere, including layers with small vertical scales. Our neglect of the horizontal component of $\nabla\theta$, an approximation valid for large Richardson number, may not apply on the smallest scales either. Fortunately, there are reasons for supposing that the large-scale dynamics 'sees' mainly a coarse-grain, spatially averaged approximation to Q and θ , varying on vertical and horizontal scales not much smaller than those resolvable by the satellite data, and moreover that many of the smaller-scale fluctuations in $\nabla\theta$ and ζ are sufficiently ill-correlated to be ignored, to a first approximation, in a coarse-grain estimate of the right-hand side of equation (1) exploiting the smoothness of the satellite weighting functions. If some such coarse-grain approximation to Q were not dynamically meaningful, numerical model simulations of the large-scale behaviour of the atmosphere would hardly be practicable. The particular stratospheric wave events under discussion were, in fact, remarkably well simulated by a high-resolution numerical forecasting model³, as far as we can tell from existing diagnostics.

We further caution that all scales are subject to ill-understood systematic errors from the algorithms used to retrieve vertical temperature profiles from IR radiances, and to errors in the radiances themselves¹. The difficulties in estimating such errors are well known, being partly due to a paucity of independent temperature measurements by other means such as rocket probes in a sufficient variety of conditions. A preliminary assessment of their effects on Q is given in ref. 2. A full investigation is beyond our present resources as it would require the careful intercomparison of all available data, including newly available satellite data on other quasi-conservative tracers such as ozone. This must be left as a major problem for the future, progress with which could eventually lead to improved data exploitation based on simultaneous estimation of the distribution of tracers and potential vorticity.

One of the main points to be made here, however, is that despite the data errors, the present coarse-grain Q maps exhibit certain qualitative features which are strikingly in accordance with expectations from well-explored lines of theoretical reasoning, supported by rational analytical models, by numerical experiments of various kinds, and by direct estimates of relevant material trajectories based on the satellite-derived wind fields. We feel justified, therefore, in claiming that those qualitative features in the Q fields to which we shall draw attention are almost certainly real, and that to this extent the data are dynamically consistent—more so, perhaps, than hitherto appreciated.

This view has received still further support from unpublished maps of another quasi-conservative quantity, ozone mixing ratio, recently obtained from the LIMS limb-scanning satellite radiometer⁴. Daily hemispheric ozone maps on the 10-mbar isobaric surface for the same period were shown to us by Professor C. B. Leovy after a first version of the present paper was circulated to colleagues and submitted for publication. This time sequence of maps shows qualitative features which, within horizontal resolution, strongly support our interpretation of the Q maps. They also provide more information in the tropics, where our estimates of Q are completely unreliable. All we can safely say about the distribution of Q in the tropics is that it must go through zero near the Equator, for reasons of dynamical stability^{5,6}.

Linear waves versus breaking waves

Two of the 850 K potential vorticity maps are shown in Fig. 2, for 17 and 27 January 1979, the same dates as in Fig. 1. It should be remembered that they are at best coarse-grain maps, that some of the smaller visible features may not be real, and that the real Q fields are likely to have unresolvable fine-grain structure. Owing to data problems in the tropics, details in the outermost contours are especially to be distrusted wherever they extend south of 20° N, the southernmost latitude circle shown. It is probably reasonable to think of the maps as resembling a blurred view of reality seen through a pane of knobbly glass, the size of the knobbles being of the order of many hundreds of kilometres, and more in the subtropics owing to the larger spacing of the satellite orbits there^{1,2}. The quantity plotted is

$$\hat{Q} = g^{-1} H_0^{-1} p_0 Q \quad (5)$$

in units of $10^{-4} \text{ K m}^{-1} \text{ s}^{-1}$, p_0 being a standard sea-level pressure taken as 1,000 mbar and H_0 a standard pressure scale height taken as 6.5 km. Values greater than 6 units are picked out by the heavy shading. To obtain a feel for the numerical values, one may note from equations (1), (4) and (5) that, for a given numerical value of $\hat{Q}$, the vertical absolute vorticity component Z_0 which would be realized if $\partial\theta/\partial p$ were brought to a standard value $\partial\theta_0/\partial p$, by means of an adiabatic, frictionless,

rearrangement of mass, satisfying equations (2) and (3), is given by

$$Z_0 = \frac{-Q}{g\partial\theta_0/\partial p} = \frac{-H_0\hat{Q}}{p_0\partial\theta_0/\partial p} \quad (6)$$

Conveniently, this equals $0.2\hat{Q}$ in units of 10^{-4} s^{-1} , for example, $2.0 \times 10^{-4} \text{ s}^{-1}$ on the contour marked 10 (which may be compared with the maximum planetary vorticity $2\Omega = 1.458 \times 10^{-4} \text{ s}^{-1}$), when $-\partial\theta_0/\partial p$ is taken as 32.5 K mbar^{-1} . The latter value happens to be close to the area average of $-\partial\theta/\partial p$ on the 850 K isentropic surface north of 45° N on 17 January, according to the satellite data analysis scheme, albeit about 15% too high for the 27 January data.

Comparing Fig. 2a with Fig. 1a, we see that the main circumpolar vortex appears to be a region of substantial cross-stream potential vorticity gradients, suggesting that it is well able to support Rossby-wave propagation. That is no more than has been shown from previous analyses using eulerian averaging around latitude circles, but Fig. 2a gives a better idea of the tightness of the gradients and their instantaneous spatial distribution. A large part of the overall gradient between equatorial and polar regions seems to be concentrated near the edge of the heavily shaded region, while systematic poleward gradients are comparatively weak throughout most of the surrounding, unshaded region. It seems unlikely that this structure is a result of radiative heating or cooling, especially when its observed time evolution is taken into consideration (S. B. Fels and D. L. Hartmann, personal communication).

A more likely explanation will emerge from the following discussion, and will suggest in turn that the gradients near the edge of the heavily shaded region may, in reality, be even tighter than they appear on the maps. Taken at face value, as they appear in Fig. 2a, the numerical values of these gradients are still quite large. Expressed as isentropic gradients of Z_0 (locally equivalent to isobaric gradients of 'quasi-geostrophic potential vorticity')⁷, they reach values exceeding the local planetary vorticity gradient $\beta = 2\Omega a^{-1} \cos \phi$ by well over an order of magnitude, over Canada and eastern Siberia for instance, a being the Earth's radius and ϕ the latitude. The largest contributor is the gradient of ζ . Gradients in $\partial\theta/\partial \ln p$ contribute in the same sense, as does the planetary vorticity gradient itself, but appear to be numerically less important for the coarse-grain Q distribution in the main vortex. (This suggests that the balance of terms discussed by Simmons⁸ should give a good first approximation to the wave structure, insofar as it can be described by linear theory.)

Outside the heavily shaded region, there are clear signs that the dynamics is less wave-like, and indeed highly nonlinear. This is suggested particularly strongly by Fig. 2b, and also by the Q maps for a number of other days, omitted for brevity. Especially notable is the gross shape of the '4' contour in Fig. 2b, picked out by the lighter shading and emphasized in the coloured version on the front cover of this issue. A long tongue of high- Q air seems to have been pulled out from the main vortex, and to be in the process of being mixed quasi-horizontally and irreversibly into the surrounding region of weaker gradients. As we shall see, this can be accounted for in terms of clockwise advection by the large secondary vortex centred north-east of the Aleutian Islands in Fig. 1b. Like the rest of the pattern, this Aleutian vortex was nearly stationary in position, although growing in size, during the previous few days. The Aleutian vortex, it appears, was eating its way into the potential vorticity gradient at the edge of the main vortex, systematically reducing the area best capable of supporting Rossby-wave propagation.

Although this erosion of the main vortex, and irreversible mixing of its material into lower latitudes, is a highly nonlinear process, quite outside the scope of linear wave theory, it is familiar from various theoretical model studies. Essentially the same process is illustrated, for instance, by the time-dependent theory of 'nonlinear critical layers'^{9,10}. An example is shown in Fig. 3, in which x corresponds to longitude west and $-y$ to

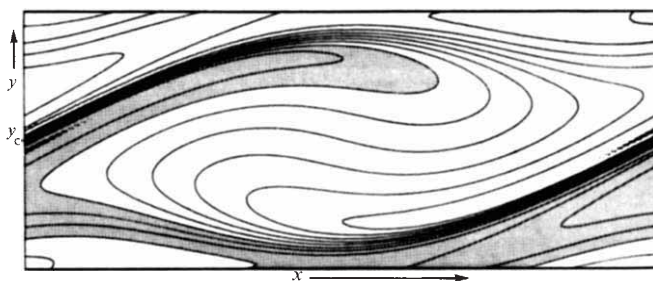


Fig. 3 Analytical solution from the time-dependent theory of nonlinear critical layers^{9,10}, exhibiting irreversible deformation of material contours due to advection by a two-dimensional (height-independent) 'Aleutian vortex' set up by a stationary Rossby wave on a shear flow. An equation of the form of equation (3) holds exactly, so that the contours are both material contours and contours of Q , or equally its two-dimensional equivalent, the vertical component of absolute vorticity. The shading picks out values of Q intermediate between the higher and lower values (unshaded) at bottom and at top/centre, respectively. The solution is periodic in x . The y scale is exaggerated. Initially the contours lie parallel to the x axis and represent a monotonic gradient of Q . The initial flow is in the x direction before the waves are excited, and its velocity is proportional to $(y - y_c)$ so that there is a 'critical line', where flow speed equals wave phase speed, at $y = y_c$. The time elapsed is 0.57 of the time for an air parcel to make one complete trip around the centre of the vortex or 'cat's eye'.

latitude (see Fig. 3 legend for further detail). This solution was obtained analytically, using the method of matched asymptotic expansions, thus avoiding any questions about numerical resolution. It provides a dynamically consistent model example of the effect of an indefinitely persistent 'Aleutian vortex' on a set of material contours, coinciding with Q contours, which initially lie parallel to the x direction. The model Aleutian vortex is twisting up the contours like spaghetti on a fork, destroying the pre-existing, overall gradient of Q . The shading in Fig. 3 emphasizes the fact that long, thin tongues of material, like that suggested by Fig. 2b, are produced by this advective process. The same process has been simulated in various kinds of numerical experiment¹⁰⁻¹⁴. Figure 3 also illustrates the well known fact that such advective processes tend to produce Q fields of increasingly fine spatial scale, exemplifying the so-called 'potential enstrophy cascade' studied in the theory of geostrophic turbulence¹⁵. This, of course, is one of the reasons that Q is likely to vary on much finer scales than could possibly be resolved by observational data.

It might still be questioned whether the tongue of high- Q air appearing in Fig. 2b is real, since for reasons already indicated there is little hope of showing this directly from the data alone, especially in view of data limitations in the subtropics. Still less is there any point in trying to make direct estimates of the advection term $\mathbf{u} \cdot \nabla Q$ on the left-hand side of equation (3), because the extra differentiation involved in estimating ∇Q would make the effect of small-scale errors even worse than in Q itself. A better check is to use satellite-derived wind fields to calculate isentropic trajectories, which apart from errors in the wind fields would represent the paths of material parcels if equation (2) were exactly satisfied. A number of such trajectories were calculated, integrating backwards in time from a selection of positions within and near the tongue as it appears in Fig. 2b. The trajectories were found to extend back around the growing Aleutian vortex to positions close to where the edge of the main vortex had been on the appropriate date. For instance, an air parcel at 177.5° W , 35° N on 27 January, near the tip of the visible tongue, was estimated to have been over the Arctic Ocean about 4 days earlier, around 80° N and close to the 4 contour at the edge of the shaded region. The trajectory crosses the north coast of mainland Canada near Victoria Island before swinging out over the Pacific via northern California. In computing this trajectory we had to correct for the error in the gradient-wind approximation due to strong deceleration of the

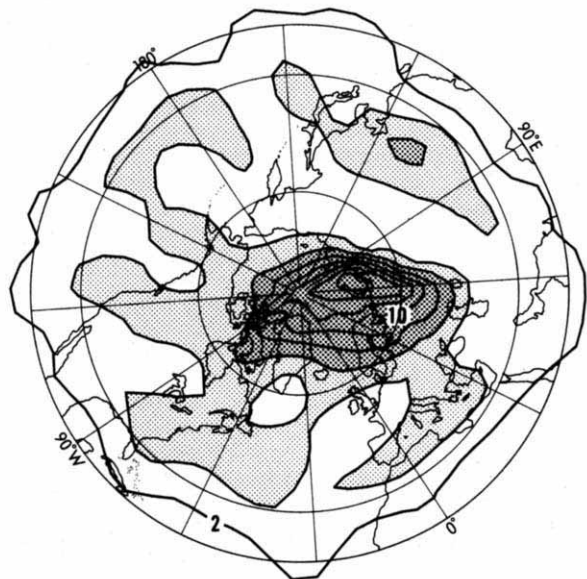


Fig. 4 Ertel's potential vorticity as in Fig. 2, but for 16 February 1979. The shading refers to same contour values as in Fig. 2. The small area of the main vortex suggests that the stratosphere was highly 'preconditioned', that is, susceptible to a major warming.

parcel as it came out of the main vortex¹⁶. Another air parcel, at 117.5° W, 35° N on 27 January, near Los Angeles, was estimated to have passed close to the North Pole about 2 days earlier, well within the shaded region at the time. Such estimates strongly suggest that advection of high- Q air from the edge of the main vortex could, indeed, have produced a thin tongue having the length, position and gross shape suggested by Fig. 2b. They suggest, moreover, that the process would have taken only a few days, so that equations (2) and (3) are likely to have been relevant despite the possible effects of radiative heating or cooling.

The phenomenon seen in Figs 2b and 3 is analogous, in a very fundamental sense, to the breaking of ocean waves approaching a beach, and it is appropriate to speak of a breaking Rossby or planetary wave. The basic criterion for saying whether a wave of any kind is breaking is whether material contours and surfaces are being irreversibly deformed, rather than simply undulating back and forth as is assumed in linear wave theory. Wave breaking, in this sense, is undoubtedly a ubiquitous phenomenon and is one of the most effective means whereby waves in naturally occurring flows can cause systematic redistribution not only of potential vorticity, but also of pollutants, angular momentum and other quantities of interest. Other significant examples include (1) the breaking, in the upper troposphere, of packets of Rossby waves radiated upwards and equatorwards by occluding tropospheric depressions¹⁷⁻¹⁹ (vital to the global potential-vorticity and angular momentum balances, but less accessible to direct observation than the present example because the spatial scales are smaller), and (2) the breaking of upward-propagating internal gravity waves in the mesosphere, revealed by noctilucent cloud patterns and by modern radar techniques, and now believed on good evidence to be the key to an old enigma about the global angular momentum balance at altitudes above 50 km (see, for example, ref. 20). Unlike potential vorticity and material tracers, momentum and angular momentum can be transferred between, and not merely within, the sites of wave generation and wave breaking. General theories of wave, mean-flow interaction imply that the distinction between breaking and non-breaking waves—defined, as here, in terms of irreversibility or reversibility of the deformation of material contours—is fundamental to all the aforementioned processes²¹. The distinction seems to be more fundamental, for instance, than questions of detail such as whether local instabilities have any role in deforming the material contours,

or whether or not the breaking can be associated with a 'critical layer' as in Fig. 3.

Although the data resolution can hardly be expected to be good enough to reveal wave breaking events of smaller scale than that in Fig. 2b, we may note in passing that there is more than a hint of such an event (as seen through 'knobbly glass') in the Z-shaped contour, labelled 2 units, near the bottom of Fig. 2a. Despite the severe data problems in this region, the feature is broadly consistent with advection by the observed velocity field during the previous few days. Perhaps more significantly, however, the theoretical considerations mentioned earlier show that the occurrence of such breaking is more or less inevitable in any case, given a few long-accepted observational facts. At most altitudes in the stratosphere, the eulerian-mean flow $\bar{u}$ around latitude circles usually vanishes in at least some part of the tropics or subtropics, as seen in a frame of reference moving longitudinally with the waves of largest amplitude. The waves are often nearly stationary, or moving slowly eastwards (as happens to be the case for the wave-3 disturbance seen in Fig. 1a), whereas $\bar{u}$ goes from large eastward values in the winter hemisphere to large westward values in the summer hemisphere. The kinematics of the situation implies the existence of secondary vortices, in the waves' frame of reference, somewhere in the tropics or subtropics, which for realistic wave amplitudes and durations are capable of twisting up material contours irreversibly in the manner illustrated by the analytical solution in Fig. 3.

We therefore conclude (1) that the region of weak potential vorticity gradients surrounding the main vortex is effectively a gigantic 'surf zone', in some part of which planetary waves are breaking most if not all the time, and (2) that the resulting quasi-horizontal mixing is a likely explanation of why the gradients in this zone are observed to be weak.

Encroachment of the surf zone

The analogy between the region surrounding the main stratospheric vortex and the surf zone on an ocean beach appears to be both fundamental and useful, but like any other partial analogy it should not be pushed too far. Two differences may be important. First, theoretical evidence has accumulated suggesting, perhaps surprisingly, that the stratospheric surf zone may be a fairly good Rossby-wave reflector in at least some of the conditions of interest (ref. 11 and refs therein). This is quite unlike an ordinary ocean beach, which is a good wave absorber. Second, whereas ocean beaches are more or less fixed in position, the stratospheric surf zone can encroach polewards whenever erosion of the main vortex is strong enough.

Figure 4, showing Q for 16 February 1979, seems to be an excellent illustration of this last point. The reduction in the heavily shaded area is unmistakable when Fig. 4 is compared with Fig. 2a. Indeed, the heavily shaded area in Fig. 4 is perhaps more appropriately compared with the lightly shaded area in Fig. 2a, if we define the area of the main vortex by reference to the outer edge of the region of tight gradients. We consider erosion due to wave breaking to be the most plausible explanation. It can simultaneously explain the reduction in the area of the main vortex, the corresponding broadening of the surrounding zone of weak gradients, and the persistently tight gradients marking the interface between them (apparent in all the available Q maps for the period). Gradients as tight as this, or even tighter, are characteristic of the sharp interface between a well mixed and a less mixed region when there is an overall gradient of some quasi-conservable quantity across the two regions. The phenomenon is familiar in several contexts, and has often been demonstrated in small-scale laboratory experiments²². The idea that vigorous quasi-horizontal mixing was occurring outside the main vortex seems consistent not only with Figs 1b and 2b but also with the entire sequence of daily Q maps for late January and early February. Most of these maps show clear signs of large-amplitude wave breaking, on a scale which seems ample to account for the observed broadening and encroachment of what we have called the surf zone.

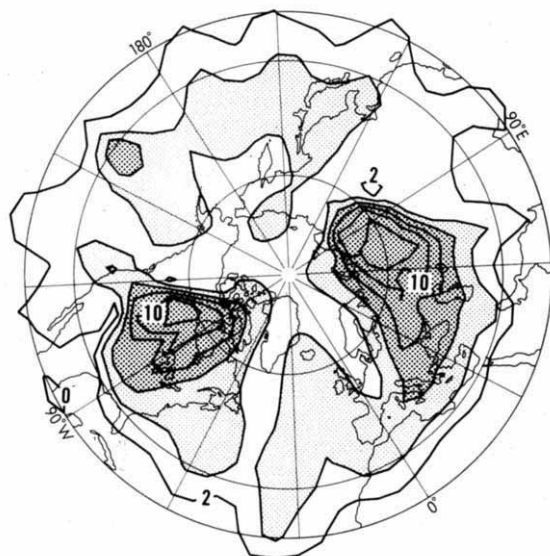


Fig. 5 Ertel's potential vorticity as in Fig. 2, but for 23 February 1979, showing the effect of the subsequent major warming.

Additional supporting evidence comes from the LIMS ozone maps shown to us by Professor Leovy. They show what seems to be the same main-vortex, surf-zone structure, evolving in time in the same way. Erosion by wave breaking can easily explain this structure and evolution because, like Q , the ozone mixing ratio is a quasi-conservative quantity in the middle stratosphere, approximately satisfying an equation of the form of equation (3) over time scales of the order of days. Alternative explanations in terms of diabatic or photochemical effects seem less likely to be successful because, although such processes cannot be expected to be negligible over the whole period in question, there is no obvious reason that they should affect ozone in the same way as Q .

Resonance, preconditioning, sudden warmings

The abovementioned differences between the stratospheric surf zone and an ordinary beach may be significant for various reasons, one of which is the much-discussed possibility¹¹ that the main vortex may behave like a resonant cavity. If so, changes in its area induced by wave breaking could provide a robust and effective mechanism for the nonlinear self-tuning or detuning of the cavity. In a recent theoretical study, Plumb²³ has pointed out that an approach to resonance via nonlinear self-tuning (that is, 'topographic instability')²⁴ can explain not only the growth of stationary wave-1 amplitude, but also the simultaneous slowing down of a weaker, westward-travelling wave-1 component in the height field, such as was observed to precede the large-amplitude wave-1 event of Fig. 1b^{25,26}. Whilst the idea of a self-tuning resonant cavity fits closely with such observations, other explanations of the apparent travelling-wave behaviour are also possible, and the very interesting theoretical questions thus raised have yet to be settled definitively.

Another significant aspect is the 'preconditioning' of the stratosphere to make it susceptible to the occurrence of a major sudden warming. From the present viewpoint, it seems natural to regard major warmings as wave events which break up the main vortex completely, advecting low potential vorticity air over the Pole along with ozone and other tracers. The accompanying descent of air parcels, due to the adjustments which maintain approximate geostrophic and hydrostatic balance, causes adiabatic compression, and hence the large polar temperature rises giving such events their customary name. This notion of a major warming as involving complete breakdown of the main vortex does not quite coincide with the definition currently adopted by the World Meteorological Organization,

but seems closer to dynamical fundamentals and is consistent, moreover, with views long held by some synoptic meteorologists²⁷. The idea that some kind of preconditioning process precedes a major warming goes back to the remarks by Quiroz, Miller and Nagatani in 1975 (see, for example, ref. 11 and refs therein), and it is now widely believed that such an idea is needed to explain many of the observed facts, including the fact that major warmings do not occur more often.

Preconditioning has often been thought of in terms of changes in the eulerian-mean state of the stratosphere, defined by taking averages around latitude circles. But it now seems evident that a much more fundamental and practically useful measure of preconditioning would be in terms of the reduction in the area of the main polar vortex, or degree of encroachment of the stratospheric surf zone. The advantage of fixing attention on the area of the main vortex is that it eliminates from consideration the large but purely temporary dynamical effects, on the eulerian mean at high latitudes, of transient, reversible disturbances to the main vortex, such as bodily migrations towards or away from the pole accompanied by little or no wave breaking. Instead, it fixes attention on the more permanent, irreversible dynamical effects, in which potential vorticity is being drastically rearranged rather than simply being moved back and forth.

We predict that unusually small values of the area A of the main vortex, as it appears on daily stratospheric potential-vorticity maps, will be found to be characteristic of the conditions observed just before major warmings. The smaller the value of A , the smaller the region best capable of supporting Rossby-wave propagation. Small values of A may thus give rise to a focusing effect, tending to concentrate any subsequent wave disturbance into a smaller and less massive region than usual (a linear model of the disturbance structure again involving Simmons' approximate balance of terms⁸). Evidence suggesting the importance of this focusing effect in the events leading to a major warming can be found both in the observational data and in numerical simulations¹¹. An objective definition of A which could be used to investigate its day-by-day behaviour is proposed in ref. 16.

Experiment in stratospheric dynamics

When the area of the main vortex is as small as Fig. 4 suggests it was on 16 February 1979, the preconditioning process is far advanced, according to our hypothesis, and a subsequent major warming very likely. In this case a major warming did occur, several days later. This warming was of exceptional interest for two reasons. The first was that the wave event precipitating it appeared to be unusually simple in form, with wave 2 dominating the eddy fluxes which measure the upward propagation of wave activity from the troposphere. Very large wave-2 amplitudes developed, splitting the main vortex cleanly in half in the course of a few days. The resulting coarse-grain potential vorticity distribution is indicated in Fig. 5, the map for 23 February. The unusual weakness of wave 1 during the splitting process provided direct evidence that large wave-1 amplitudes are not essential to that process¹¹.

The second reason that this particular warming was especially interesting was the fact that it did not occur sooner. On the basis of past case studies, one might well have expected a major warming to have occurred by mid-February²⁵. In this case, however, the preconditioning process was unusually well separated in time from the warming itself. These circumstances, the lateness of the warming and the unusually simple wave structure when it did occur, combined to make it one of the most useful 'controlled experiments' in stratospheric dynamics ever performed by the real atmosphere. This has already been taken advantage of in computer simulations which have successfully 'repeated' the experiment, and also varied its conditions in several different ways^{3,28}. The possibilities of such numerical experiments are still far from being exhausted.

Most of the other major warmings observed during the past two decades involved large-amplitude, complicated-looking

mixtures of waves 1, 2 and higher. However, drawing on what has been learned from the case of January–February 1979, we may anticipate that much of this apparent complexity will disappear as soon as attention is focused on the main vortex as the dynamical centre of things, rather than on any analysis tied to latitude circles, such as the conventional Fourier analysis of the height and temperature fields into separate wavenumbers. For instance, in the celebrated major warming of January 1977, the main vortex split in much the same way as in February 1979, the only difference being that it happened to be displaced well away from the Pole at the time, over Baffin Island²⁹. Only the Fourier analysis was complicated, not, it seems, the physical phenomenon. Regarded as a wave-2 disturbance relative to the displaced main vortex³⁰, rather than relative to latitude circles, the observed phenomenon appears remarkably similar to that of February 1979. Indeed, the whole sequence of events leading to the warming, and no doubt those leading to other major warmings, can probably be viewed in essentially the same simple way as above, the main difference being that the preconditioning process (erosion of the main vortex) may not be well separated in time from the subsequent major warming. We await with interest the potential vorticity maps which will help to confirm or disprove these suggestions.

Conclusion

Our understanding of large-scale dynamical and eddy transport processes in the atmosphere would be greatly improved if daily isentropic maps of Ertel's potential vorticity Q were to become available on a routine basis. For example, they would increase our ability to judge the relevance of numerical model experiments and of theoretical concepts such as 'wave propagation', 'instability', 'critical layers' and so on. The above examples from the middle stratosphere seem sufficient to show that the effort would be worthwhile, despite the impossibility of resolving the finest scales in the potential vorticity patterns.

The coarse-grain Q maps presented here used nothing more than standard data-processing techniques applied to routinely-available meteorological data, but besides sharpening our insights into stratospheric dynamics in general, they have already suggested some specific, testable hypotheses. One such hypothesis is that large-scale wave breaking, leading to erosion of the main circumpolar vortex, will be found to be characteristic of the circumstances leading to major stratospheric warmings. The existence of long, thin tongues of material coming off the edge of the main vortex, a process which we believe we are seeing for the first time in Fig. 2b albeit in a blurred and distorted form, may soon be independently verifiable in this and other cases by means of data now becoming available from the newest satellite radiometers. An additional check may come from Q maps and air-parcel trajectories derived from high-resolution numerical simulations. For instance, the latest numerical forecasting models have far better horizontal resolution than data from polar-orbiting satellites, and should be able to represent a feature like the long tongue in Fig. 2b in considerable detail.

The prediction that A , the area of the main vortex on a given day, will be found to decrease systematically during the buildup to a major warming, should be testable even with data whose horizontal resolution is too coarse to show wave-breaking details. For this purpose the only demand on the data is that an objective measure of A be feasible¹⁶, based on the anticipated tightness of isentropic gradients of Q near the edge of the main vortex. If time series of daily values of A , on some convenient isentropic surface, could be obtained from long, homogeneous runs of data, then other possibilities would be opened up. Such time series should be very effective as indicators of the general state of the stratosphere during a succession of winters. They represent a good combination of simplicity and finesse, since the use of daily estimates of A avoids the loss of information incurred by taking eulerian space or time means. Time series

of A might substantially aid current attempts to correlate the interannual variability of the winter stratosphere with other long-term variations such as the quasi-biennial oscillation in the tropical stratosphere, or El Niño and the Southern Oscillation in the troposphere.

Assuming that our findings are confirmed, they also have implications for the next generation of stratospheric tracer transport models, with which the possible effects of pollutants on the ozone layer will be studied. Short of attempting to simulate the three-dimensional motion of the entire troposphere, stratosphere and above³¹, some success has already been achieved with 'two-dimensional' models in which the material transports are represented by means of eulerian eddy fluxes relative to latitude circles. A number of significant suggestions for improving models of this kind have recently been made^{32–36}, and the observational picture now emerging should be a useful input to such modelling efforts. Indeed, it suggests that the most efficient two-dimensional modelling strategy might, in fact, be to abandon altogether the description tied to latitude circles, and instead to partition each isentropic layer in the stratosphere into several regions on the basis of the coarse-grain distribution of Q —say, a main vortex, a surrounding surf zone, and other regions representing the tropics and the summer hemisphere. From a theoretical point of view, this would amount to a low-resolution 'modified lagrangian-mean description'³⁷ of the stratosphere. The mass of each region would change with time according to observational estimates of erosion rates, in competition with the rate of replenishment of potential vorticity by diabatic processes such as IR cooling in the polar night. The detailed implementation of such a model has yet to be worked out.

For the troposphere, the best hope of obtaining the needed Q maps may be to use dynamically consistent 'data' derived from high-resolution numerical weather forecasting models and analysis schemes, rather than making any attempt to use raw observational data. We may anticipate that Q maps produced as part of the daily numerical forecasting operation will prove valuable in the routine assessment of the forecasts themselves, since they make the model dynamics highly visible. For instance, evidence now becoming available suggests that such maps will lead to immediate insights into the formation and maintenance of the mid-latitude 'blocking' situations which lead to anomalous spells of weather (B. J. Hoskins, G. J. Shutts, personal communication; see also refs 38, 39). They would also make it easier to see what dynamical instability mechanisms are likely to be operating in a given locality. Another promising area concerns the interaction between middle latitudes and the subtropics, one aspect of which is the reflectivity of the subtropics to the packets of Rossby waves radiated upwards and equatorwards by occluding mid-latitude depressions^{17–19}. The theory of nonlinear critical layers suggests that this reflectivity, whose effects could be important for medium-range weather forecasting, will be dependent from day to day on the distribution of Q on isentropic surfaces in the high troposphere equatorward of the subtropical jet, where the waves may be expected to break.

A longer version of this article, with more figures and a more complete bibliography, is to appear as ref. 16. An extensive bibliography and background discussion may also be found in a recent review article by one of us¹¹.

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Note added in proof: For related work on large-scale ocean dynamics, see ref. 40.

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Inhibition of RNA cleavage but not polyadenylation by a point mutation in mRNA 3' consensus sequence AAUAAA

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A single U → G transversion in the 3' consensus sequence AAUAAA of the adenovirus early region 1A gene was constructed and the effect of this mutation on processing of the 3' end of the nuclear early region 1A RNAs was analysed. The results demonstrate that the intact AAUAAA is not required for RNA polyadenylation but is required for the cleavage step preceding polyadenylation to occur efficiently.

THE AAUAAA hexanucleotide and closely related sequences are the only primary structures common to the 3' ends of mRNAs in higher eukaryotes, excluding histone mRNAs¹. Because most non-histone mRNAs are polyadenylated, this observation led to the suggestion^{1,2} that AAUAAA is part of a recognition signal required for proper processing and polyadenylation of eukaryotic mRNAs. This was confirmed by Fitzgerald and Shenk³, who showed that in a Simian virus 40 (SV40) mutant, deletion of the entire AAUAAA sequence shifted polyadenylation to just downstream from the next most proximal AAUAAA. The mechanism of polyadenylation in higher eukaryotes has not been established. However, it has been shown that in the synthesis of papovavirus⁴⁻⁷, adenovirus⁸⁻¹⁴ and several cellular mRNAs¹⁵⁻¹⁶, transcription proceeds beyond the polyadenylation site of the mature message. An endonucleolytic cleavage probably occurs in these primary transcripts at the site of polyadenylation to generate a substrate for a poly(A) polymerase which polymerizes adenylate residues onto the 3' hydroxyl group of polynucleotides^{13,14,17-21}. To explore the function of the AAUAAA sequence in the RNA cleavage and polyadenylation steps, we constructed a point mutation in this hexanucleotide in an adenovirus 2 (Ad2) transcription unit, E1A and analysed the effects of the mutation on processing of the 3' ends of E1A nuclear transcripts.

The mRNAs synthesized from early region 1A (E1A) of adenovirus 2 are typical of mRNAs in higher eukaryotes; they are spliced²²⁻²⁴, and contain a 5'-terminal cap²⁵, noncoding regions at both the 5' and 3' ends and a poly(A) tail²⁶. Figure 1 shows the sequence of the distal portion of the 3'-untranslated sequence encompassing the AAUAAA sequence and extending to the previously reported polyadenylation site near nucleotide 1,630 (ref. 26). Although some variants of this hexanucleotide have been reported²⁷⁻³⁰, the U in the third position seems to

be completely conserved. Therefore, we constructed an adenovirus mutant having a T → G transversion at this position in the DNA sequence.

RNA cleavage and polyadenylation

The T in the third position of the adenovirus E1A AATAAA sequence was changed to a G by oligonucleotide-directed mutagenesis of an M13-E1A clone³¹⁻³⁴. The mutation was transferred into the adenovirus genome³⁵ to generate a mutant virus designated Ad2pm1610 (pm1610 = point mutation at nucleotide 1,610 in the Ad2 sequence³⁶).

To analyse the effect of the point mutation on the processing of the 3' ends of the E1A nuclear RNAs (nRNAs), we prepared nRNA from HeLa cells¹¹ infected with either wild-type Ad2 or Ad2pm1610. Total nRNA was fractionated into poly(A)⁺ and poly(A)⁻ RNA (that is, poly(A) tails shorter than 15 residues) by repeated passages over oligo(dT)-cellulose columns³⁷ and then analysed by the hybridization/S₁ nuclease method³⁸. In this technique the RNA is hybridized to ³²P-labelled DNA probes in DNA excess, digested with S₁ nuclease to hydrolyse the single-stranded RNA and DNA and the S₁-protected RNA/DNA hybrid fragments are denatured and fractionated on polyacrylamide gels. As the hybridizations are performed in DNA excess and proceed to near completion, the intensities of the S₁-protected bands seen in the autoradiograms are a measure of the concentration of RNA in the analyses.

Figure 2a shows the S₁-protected fragments resulting from hybridization of the Ad2 E1A nRNAs to a DNA probe 3' end labelled with ³²P at 1,337 in the Ad2 sequence. This probe (probe A, Fig. 2d) is labelled near the 3' end of E1A and continues past the E1A polyadenylation site to include the 5' end of the next transcription unit, E1B. The major 290-nucleotide band seen in the analysis of Ad2 nRNA is protected by the

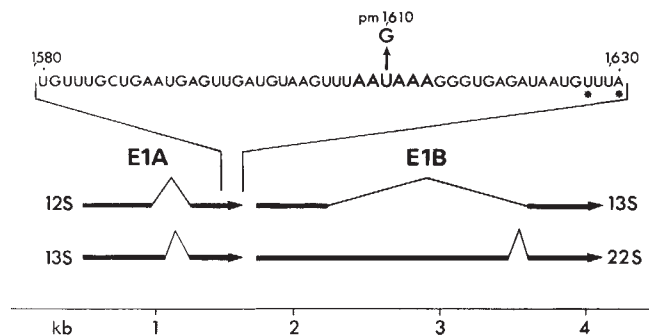


Fig. 1 Structures of the predominant E1A and E1B mRNAs at early times, and construction of Ad2pm1610. The left end of the Ad2 genome is represented by the thin line demarcated in kilobase pairs (kb). The horizontal lines joined by the carot symbols indicate the exons of the E1A and E1B mRNAs. The arrowheads indicate the direction of transcription. The sequence of the distal portion of the E1A mRNAs is shown²⁶ extending from nucleotide 1,580 to the polyadenylation sites, indicated by asterisks below nucleotides 1,627 and 1,630. The U → G transversion at nucleotide 1,610 is indicated.

Methods: The point mutation was constructed by oligonucleotide-directed mutagenesis using a 5'-CCCTTCTTAAA-3' dodecanucleotide and M13GG₁ (ref. 34) as the single-stranded E1A template. The underlined C in the dodecanucleotide corresponds to the base pair mismatch at nucleotide 1,610. The oligonucleotide was synthesized⁶³ and mutagenesis performed³¹⁻³⁴ as described previously except that no enrichments for the mutant were performed. The desired mutant, M13GG₁pm1610, was identified by dideoxynucleotide sequencing using the C reaction only. Bacteriophage DNA was amplified from 60 plaques in 10 ml tryptone yeast extract broth each. Phage were precipitated once with polyethylene glycol⁶⁴, resuspended in 0.5 ml TE buffer (10 mM Tris pH 8.0, 1 mM EDTA), phenol-extracted once, ethanol-precipitated and resuspended in 60 µl TE. Sequencing was performed with 1 µl from each sample. One out of 60 plaques screened had the base change in addition to an equal proportion of wild-type DNA. The mutant, M13GG₁pm1610, was plaque-purified and replicative form DNA prepared⁶⁴. The mutation was transferred into the Ad2 genome according to a strategy previously described³⁵. The Ad2 DNA-protein complex was prepared and digested with exonuclease III, creating single-stranded termini ~3,500 bases long³⁵. 7 µg of double-stranded M13GG₁pm1610 was digested with *KpnI* (BRL), alkali-denatured and hybridized to 2.5 µg Ad2 DNA-protein complex as described elsewhere³⁵. The hybridization products were transfected into 293 cells by the calcium phosphate technique⁶⁵. To identify the desired mutant, Ad2pm1610, DNAs were prepared from nine well isolated plaques, digested to ~3,500 bases with exonuclease III and sequenced by the dideoxynucleotide method^{41,42}. The DNA from three of nine plaques screened contained the mutation at nucleotide 1,610. Ad2pm1610 was collected from a well isolated plaque and plaque-purified a second time on 293 cells before preparing a large virus stock from suspension HeLa cells. The HeLa cell/293 titre for Ad2pm1610 is similar to that for Ad2.

3' end of the E1A nRNAs. Based on the intensities of the 290-nucleotide bands in the poly(A)⁺ and poly(A)⁻ lanes, we estimate by quantitative densitometry that 85–90% of the E1A nRNA in Ad2 is polyadenylated. The less prominent 710- and 260-nucleotide bands are protected by E1A nRNA fused to the next transcription unit, E1B (1AB RNAs, see below). These fused 1AB RNAs represent 30% of the nuclear transcripts initiated at the E1A cap site in Ad2. The other minor bands seen in the poly(A)⁺ track are due to incomplete S₁ digestion of the DNA probe and are not protected by RNA because they were also observed in a control using the DNA probe without RNA (not shown) and were not observed in the analysis of total nuclear RNA from which the poly(A)⁺ RNA was selected.

Any one of four possible effects might be anticipated as a result of the U → G transversion in the AAUAAA sequence. The mutation might: (1) have no effect on RNA processing, (2) disrupt only RNA cleavage, (3) affect only polyadenylation, (4) alter the efficiency of both RNA cleavage and polyadenylation.

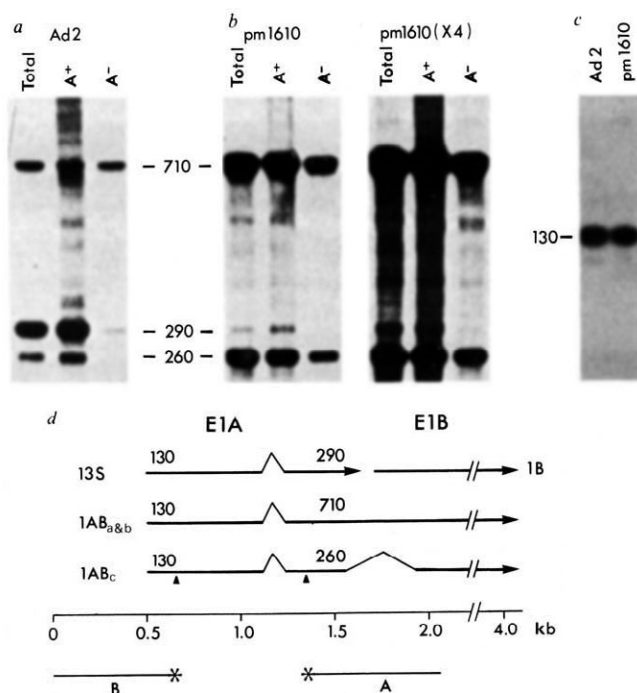


Fig. 2 S₁ analyses of the E1A nuclear RNA from Ad2 and pm1610. *a*, S₁ analysis of the 3' ends of E1A nRNAs from Ad2. *b*, S₁ analysis of the 3' ends of the E1A nRNA from pm1610. A fourfold longer exposure of the pm1610 autoradiogram is included to the right. The S₁ analyses shown in *a* and *b* were performed with a DNA probe 3' end labelled with ³²P (probe A) shown in *d* (see below). The sizes of the S₁-protected fragments are indicated in nucleotides. *c*, Comparison of the concentration of total Ad2 and pm1610 nRNA. The S₁ study was performed using total nuclear RNA and a DNA probe 5' end labelled with ³²P (probe B) depicted in *d*. The size of the major S₁-protected fragment is indicated. *d*, Interpretation of the S₁ data. The left end of the Ad2 genome is represented by the long continuous line demarcated in kilobase pairs. The DNA probes used in the S₁ studies are represented by the two lower lines, with an asterisk on the left for the 3' end labelled probe (probe A) and on the right for the 5' end-labelled probe (probe B). The lines above the Ad2 genome represent the exons of the 13S spliced forms of the E1A nRNA and the left end of the E1B and 1AB RNAs. For simplicity the 12S spliced forms of the E1A nRNAs are not represented, but were present. The two arrowheads below the 1AB_c RNA indicate the position of the ³²P end label in the two probes relative to the nRNA. The sizes of the S₁-protected fragments generated with probes A and B with the various RNAs are indicated above the appropriate RNA species.

Methods: log phase suspension cultures of HeLa cells at 4 × 10⁵ cells ml⁻¹ were infected with 10 plaque-forming units per cell and incubated for 6 h at 37 °C. Nuclear RNA was prepared as described elsewhere¹¹ except that the guanidinium-ethanol-precipitated RNA was collected at 0 °C for 20 min at 3,000 r.p.m. in an IEC centrifuge and resuspended in 3.5 M urea, 0.175 M NaCl, 5 mM Tris (pH 7.4), 5 mM EDTA and 0.5% SDS before phenol-chloroform extraction. Poly(A)⁺ nRNA was fractionated from total nRNA by three passages over oligo(dT)-cellulose columns³⁷. Nuclear RNA which does not bind oligo(dT) is designated as poly(A)⁻, although these RNAs may have poly(A) tails shorter than 15 residues. The hybridization/S₁ nuclease technique was performed as described previously³⁸ with probes A and B. Probe A was prepared by digesting M13GG₁ and M13GG₁pm1610 RF (homologous with Ad2 and pm1610 RNA, respectively) with *XbaI* and 3' end labelled using [α-³²P]dATP and reverse transcriptase (Life Sciences Inc.). Probe B was prepared by T. F. Osborne by purifying the terminal Ad2 *Sau3A* fragment (1–630 base pairs) and 5' end labelling with [γ-³²P]ATP and T4 polynucleotide kinase. Hybridizations were with RNA from 2 × 10⁷ cells per lane for 12 h at 53 °C with probe A or 44 °C with probe B. The S₁-protected products were fractionated by electrophoresis on 16-cm 8 M urea-5% polyacrylamide gels.

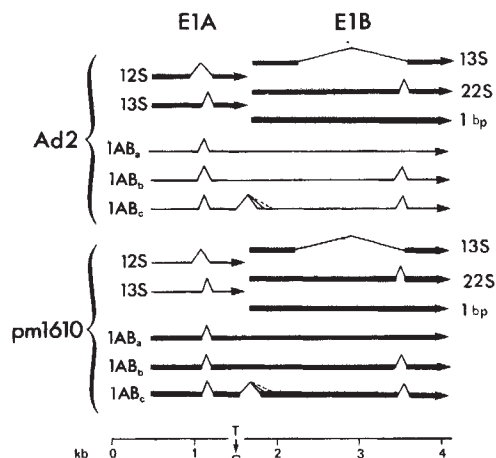


Fig. 4 Structures of poly(A)⁺ nuclear RNAs from E1A and E1B of Ad2 and pm1610. The bottom line represents the left end of the 36-kb Ad2 genome demarcated in kilobase pairs. The position of the T→G transversion in pm1610 is shown. The lines joined by the carot symbols above the Ad2 genome depict the exons of the nuclear RNA structures from E1A and E1B. The arrowheads show the direction of transcription. RNAs which constitute greater than 15% of the total poly(A)⁺ transcripts from E1A or E1B are indicated by the bold lines. For simplicity, only those 1AB RNAs spliced at the 13S splice site in E1A are shown, but 1AB RNAs with the 12S E1A splice also occur. The positions of the two major and several minor 3' cryptic splice sites in 1AB_c are indicated by the solid and broken lines on the right side of the middle carot symbol.

No shift in the E1A poly(A) addition sites was detected in pm1610 nRNA (Fig. 3). Therefore, the mutation in the AAUAAA sequence affects only the efficiency and not the accuracy of RNA cleavage.

Fused 1AB RNAs utilize cryptic splice sites

The remaining 97% of the pm1610 RNAs not cleaved at the 3' end of E1A extended into the next transcription unit, E1B. The structures of these fused 1AB RNAs shown in Fig. 4 were mapped by the hybridization/S₁ nuclease technique using a variety of 5' and 3' end labelled and uniformly labelled DNA probes (for example, Fig. 5a, b, and data not shown) and by sequencing the 3' end of the E1A RNAs (Fig. 3). All three 1AB RNAs (Fig. 4) accumulated in the nucleus. However, the 1AB_a RNA, spliced only in E1A, appeared predominantly in the nucleus (2,720-nucleotide band, Fig. 5a) and may be a precursor of the pm1610 nuclear 1AB_b and 1AB_c RNAs also found in the cytoplasm which were spliced in both E1A and E1B (Fig. 4). The 1AB_c RNAs (Fig. 4) have undergone an additional splice which cannot occur in E1A or E1B RNAs. This splice extends from nucleotide 1,599 near the 3' end of E1A to several cryptic 3' splice sites near the 5' end of E1B (Fig. 5b).

The 5' and two major 3' cryptic splice sites were mapped to nucleotides 1,599, 1,921 and 1,948, respectively, based on the sizes of the S₁-protected bands and homologies to known consensus splice site sequences⁴³. Kitchingman *et al.*²⁴ observed by electron microscopy a single 1AB nuclear transcript from Ad2 with an intron mapping closely to the position of the middle intron in 1AB_c. Additional evidence that the S₁-sensitive site at nucleotide 1,599 is generated by RNA splicing rather than RNA cleavage was obtained by sequencing the E1A RNAs (Fig. 3). If the S₁-sensitive site at nucleotide 1,599 was due to RNA cleavage and polyadenylation, then a poly(A) tract starting at nucleotide 1,599 would be visualized on the sequencing gel of poly(A)⁺ RNA; however, no poly(A) tract begins at this nucleotide.

No transcription termination sites

Our results indicate that there is no specific transcription termination site between E1A and the 3' end of E1B. The 1AB

nRNAs that were not cleaved at the end of E1A were either polyadenylated at the E1B poly(A) addition site at nucleotide 4,061, generating the 2,720-nucleotide band in Fig. 5a, or were poly(A)⁻ and had heterogeneous 3' ends (data not shown). We presume that these poly(A)⁻ nRNAs with heterogeneous 3' ends within E1B represent nascent transcripts. Thus, no RNA accumulates with a 3' end suggestive of a stable terminated molecule between the 3' end of E1A and the 3' end of E1B. There also seems to be no specific transcription termination site within 1,500 nucleotides beyond the E1B polyadenylation site. Most (>98%) of the E1B RNA cleaved at nucleotide 4,061 was polyadenylated (430-nucleotide band, Fig. 6a; 1,260-nucleotide band, Fig. 6b). However, the E1B poly(A)⁻ RNA had either heterogeneous ends or extended at least 1,500 nucleotides beyond the E1B polyadenylation site, so that it protected the full length of Ad2 sequence in probe E, Fig. 6 (2,000-nucleotide band, Fig. 6a). Thus, although E1A RNAs initiated at nucleotide 498 are normally cleaved and polyadenylated at nucleotides 1,627–1,630, the RNA polymerase probably continues to transcribe at least an additional 4,000 nucleotides. It is possible, however, that there is a specific RNA termination site for the E1A RNAs but that the termination occurs only after an RNA cleavage site has been selected.

Polyadenylation and RNA splicing

Various biological functions have been suggested for polyadenylation, although no roles have been unambiguously ascribed^{44,45}. As polyadenylation frequently precedes the RNA splicing event^{9,15,46–48} and histone mRNAs usually lack both introns and poly(A) tails^{44,45,49,50}, it has been proposed that polyadenylation may be required for RNA splicing⁵¹. Examination of the E1B and 1AB RNAs is not consistent with this hypothesis. Approximately 10% of the E1B RNA spliced at the 22S splice site fails to bind to oligo(dT)-cellulose (700-nucleotide band, Fig. 6b). These RNAs which fail to bind oligo(dT)-cellulose represent poly(A)⁻ species and are not due to inefficient poly(A) selection because >98% of the properly cleaved E1B RNA bound to the oligo(dT)-cellulose (1,260-nucleotide band, Fig. 6b). We have also found that almost all the 1AB RNAs which fail to bind oligo(dT)-cellulose have heterogeneous 3' ends and have undergone at least one of the three potential RNA splicing events (data not shown). Therefore, poly(A) tails longer than 15 nucleotides are not required for RNA splicing. This is in agreement with earlier studies of late Ad2 nRNA and more recent studies using cordycepin, an inhibitor of poly(A) polymerization^{39,52}.

Discussion

We have found that a single U→G transversion in the AAUAAA hexanucleotide of the E1A mRNAs of adenovirus decreases the efficiency of RNA cleavage of the primary transcript. However, the mutation does not diminish the efficiency of polyadenylation of those molecules which are cleaved. Our interpretation is that the intact AAUAAA sequence is required for efficient RNA cleavage, but not for polyadenylation. Perhaps bases in the AAUAAA hexanucleotide other than the U are required for polyadenylation. Alternatively, polyadenylation may simply be tightly coupled to RNA cleavage so that the site of polyadenylation is determined by the endonuclease. This is consistent with our failure to detect a significant amount of nuclear RNA which is cleaved but not polyadenylated. Furthermore, in studies of *in vitro* RNA processing in isolated nuclei where cleavage and polyadenylation are relatively inefficient, Manley *et al.*^{14,20} found that all RNA which was cleaved at a poly(A) site was also polyadenylated. Tight coupling between RNA cleavage and polyadenylation could explain why histone mRNAs in higher eukaryotes are not polyadenylated, as it appears that the 3' end of histone mRNAs are generated by transcription termination rather than RNA cleavage⁵³.

The AAUAAA hexanucleotide alone cannot be the entire RNA cleavage signal because AAUAAA sequences have been observed at internal positions distant from the 3' ends of mRNAs

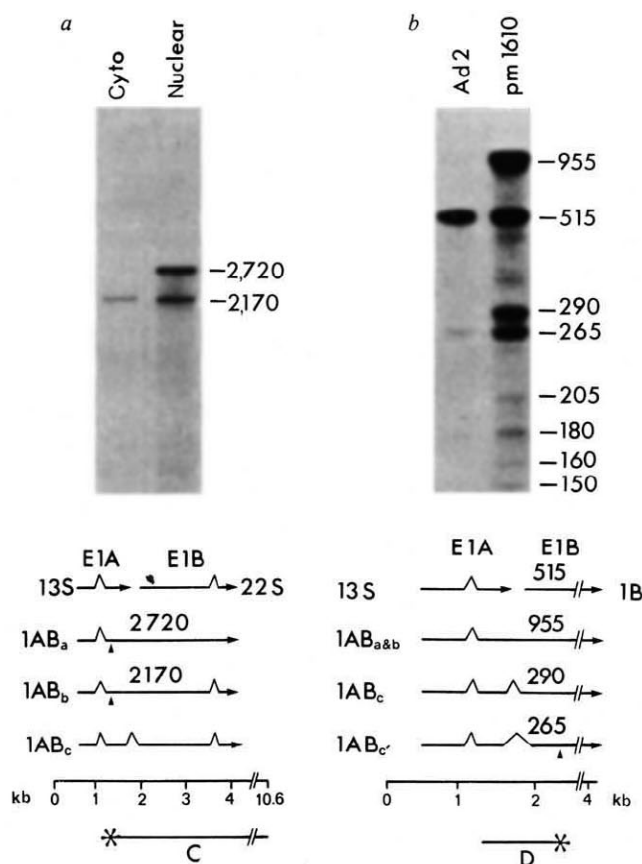


Fig. 5 Mapping the structures of the 1AB RNAs by the S_1 method. *a*, Mapping the 3' end of the 1AB RNAs; *b*, mapping the cryptic 3' splice sites in the 1AB RNAs. The sizes of the S_1 -protected fragments are indicated in nucleotides. The 3' and 5' 32 P end-labelled DNA probes (probes C and D, respectively) are represented by the bottom line in the figures below the corresponding autoradiogram. The line above the probe represents the left end of the Ad2 genome. The structures of the 1AB RNAs including the 13S E1A and the 22S E1B RNAs are indicated.

Methods: Probe C was prepared by digesting 1.6 μ g Ad2 DNA with *Xba*I and 3' end labelling with [α - 32 P]dCTP and reverse transcriptase. The 9-kb *Xba*I B fragment was purified from an agarose gel as described previously⁶⁸. Probe D was prepared by digesting 3 μ g pBE5 (ref. 69) with *Ava*I, 5' end labelling with [γ - 32 P]ATP and T4 polynucleotide kinase and eluting the 956-base pair fragment (nucleotides 1,257–2,212) from a 5% polyacrylamide gel. Cytoplasmic RNA was prepared as previously described¹¹ from suspension culture HeLa cells 6 h post-infection at a multiplicity of infection of 10. Hybridization with probe C was for 3 h at 55 °C with total cytoplasmic RNA from 1×10^7 cells or nuclear poly(A)⁺ RNA from 4×10^7 cells. Hybridization with probe D was for 14 h at 51 °C with total nRNA from 1×10^7 cells. The S_1 -protected products were fractionated by electrophoresis on 16-cm 8 M urea–5% polyacrylamide gels.

(see, for example, refs 29, 54–57). Because no other sequence occurs universally at the 3' ends of mRNAs, a specific RNA secondary or tertiary structure may be the additional feature of the RNA cleavage signal.

Signals separate from the hexanucleotide may also determine the distance between the AAUAAA sequence and the poly(A) site. Despite the base pair change in the AAUAAA sequence, the same E1A polyadenylation sites are used by pm1610 and Ad2. One SV40 mutant with deletions flanking but not including the AAUAAA hexanucleotide produced RNA having an altered distance between the AAUAAA sequence and the polyadenylation site³. The accuracy of RNA cleavage may also be controlled by signals outside the AAUAAA sequence. RNAs with normal AAUAAA hexanucleotides display varying degrees of microheterogeneity in the choice of poly(A) addition sites^{3,58,59}. In pm1610, the U→G base change decreased the

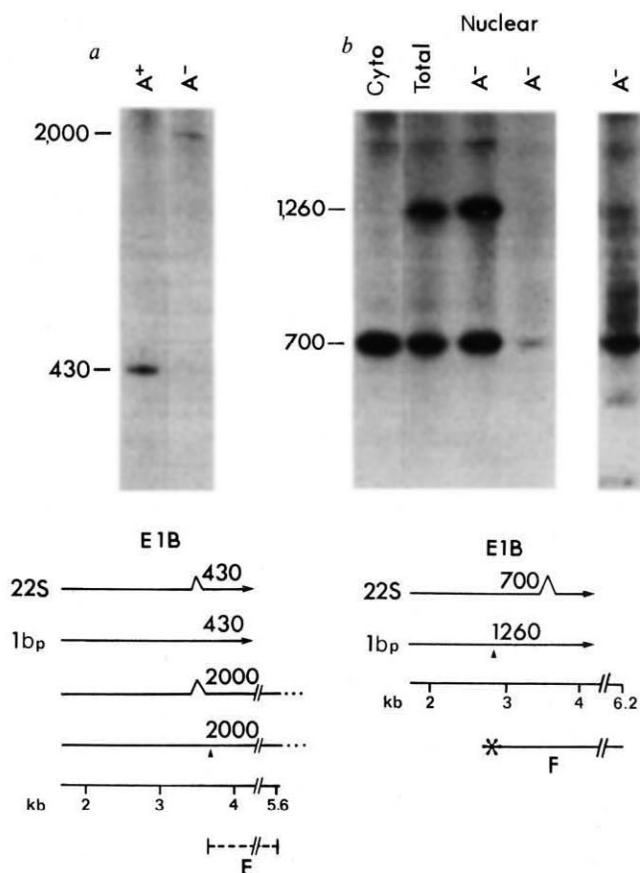


Fig. 6 S_1 analyses of the 3' end of the E1B RNAs. *a*, mapping the 3' end of the E1B poly(A)⁻ nRNA from Ad2. S_1 analysis was performed with the uniformly 32 P-labelled DNA probe (E) indicated by the broken line in the figure below the autoradiogram. The sizes of the S_1 -protected fragments are shown in nucleotides. *b*, Identification of spliced E1B nRNA from Ad2 which is poly(A)⁻. S_1 analysis was performed with the DNA probe (F) 3' end labelled with 32 P depicted by the bottom line in the figure below the autoradiogram. The lengths of the S_1 -protected bands are given in nucleotides. A longer exposure of the nuclear poly(A)⁻ track is included to the right.

Methods: Probe E was constructed by cloning the r strand of the *Sst*I D fragment from Ad2 (nucleotides 3,633–5,634)³⁶ into the *Sst*I site of M13Gori1 (ref. 64) (M131BPA). M131BPA DNA was 32 P-labelled *in vivo* as described elsewhere⁷⁰ to produce a uniformly labelled single-stranded DNA probe. Probe F was prepared by digesting 1.2 μ g pH2 (HindIII C fragment from Ad2³⁶ cloned into the HindIII site of pBR322) with HindIII and 3' end labelling with [α - 32 P]dATP and reverse transcriptase. Ad2 nuclear poly(A)⁺ or poly(A)⁻ RNA from 5×10^6 cells was hybridized to probe E and digested with S_1 nuclease as described previously³⁴. Ad2 cytoplasmic and nuclear RNA from 7×10^6 and 2×10^7 cells, respectively, were hybridized to probe F for 5 h at 57 °C. The S_1 -protected fragments were fractionated on 8 M urea–5% polyacrylamide gels.

rate of RNA cleavage, but did not affect the microheterogeneity of the cleavage site.

Although it is generally accepted that the 3' ends of mRNAs are generated by endonucleolytic cleavage this has not been absolutely established. If the 3' ends of the E1A RNAs are created by RNA cleavage we might expect to see another nRNA species whose 5' end is generated by the cleavage. However, we see no evidence for such a product. Perhaps this species is formed but rapidly degraded due to the absence of a 5'-terminal cap structure⁶⁰. Alternatively the 3' end of mRNAs may be created by a very rapid exonuclease which removes the ends of the mRNA precursor. Although our results do not rule out this latter possibility, they are more consistent with the cleavage hypotheses because the mutation results in increased production of fused 1AB RNAs.

Unlike the situation in higher eukaryotes, most yeast mRNAs lack an AAUAAA sequence and transcription termination seems to occur at the 3' end of the mRNAs following a T-rich consensus sequence described by Zaret and Sherman⁶¹. For these yeast mRNAs, polyadenylation may be coupled to transcription termination rather than RNA cleavage. In higher eukaryotes it has become increasingly clear that the 3' end of mRNAs are usually, if not always, generated by endonucleolytic cleavage. However, little is known about transcription termination. Transcription termination has been localized to regions beyond the 3' ends of the mouse major β -globin mRNA⁶² and

the major late adenovirus mRNAs^{9,10,14}. However, it is still unclear whether transcription terminates at specific sequences. Our results indicate that there is no transcription termination site between the 3' end of E1A and 1,500 bases beyond the 3' end of E1B.

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Structural and immunological similarities between simian sarcoma virus gene product(s) and human platelet-derived growth factor

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The predicted amino acid sequence of the simian sarcoma virus (SSV) transforming gene product, p28^{sis}, closely corresponds to that of human platelet-derived growth factor (PDGF). We demonstrate that p28^{sis} rapidly undergoes a series of discrete processing steps including dimer formation and proteolytic digestion to yield molecules structurally and immunologically resembling biologically active PDGF.

ACUTE transforming retroviruses have arisen in nature by substitution of viral genes necessary for replication with discrete segments of host genetic information¹. When incorporated within the retroviral genome, such transduced cellular sequences, termed *onc* genes, acquire the ability to induce neoplastic transformation. Some of these same cellular genes or proto-oncogenes have been implicated as important targets for genetic alterations that may lead normal cells to become malignant²⁻¹⁰. Despite great strides in identifying cellular genes with transform-

ing potential, little is yet known about proto-oncogene function or how the altered counterparts of these genes disrupt normal growth regulation.

Very recent findings have provided the first direct link between an *onc* gene and a known biological function. The simian sarcoma virus (SSV) *onc* gene, *v-sis*, has been sequenced¹¹ and its 28,000 molecular weight (MW) product, p28^{sis}, identified by means of antisera prepared against small peptides derived from sequence analysis of *v-sis*¹². Studies on

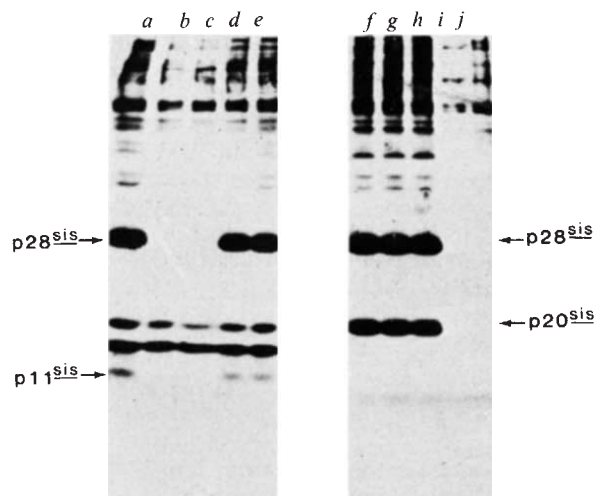


Fig. 1 Identification of 20,000 and 11,000 MW SSV transforming gene products. Subconfluent cultures containing around 10^7 cells per 10-cm Petri dish were labelled for 3 h at 37 °C with 4 ml of methionine- and cysteine-free Dulbecco's modified Eagle's minimal essential medium (DMEM) containing 100 μ Ci of 35 S-methionine and 100 μ Ci of 35 S-cysteine ($\sim 1,200$ Ci mmol^{-1} ; Amersham) per ml. Labelled cells were lysed with 1 ml of a buffer containing 10 mM sodium phosphate, pH 7.5, 100 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS and 0.1 mM phenylmethylsulphonyl fluoride, clarified at 100,000 g for 30 min and divided into four aliquots. Each aliquot was incubated with 3 μ l of antiserum for 60 min at 4 °C. Immunoprecipitates were recovered with the aid of *Staphylococcus aureus* protein A bound to Sepharose beads (Pharmacia) and analysed by electrophoresis in SDS 14% polyacrylamide gels (PAGE)¹². Extracts of labelled SSV(SSAV) transformed producer marmoset cells (HF/SSV) were immunoprecipitated with anti-*sis*-N (lanes a-e) or anti-*sis*-C (lanes f-j) serum. In some cases antibodies were incubated prior to immunoprecipitation with 1 μ g (lanes b, g) or 5 μ g (lanes c and h) of *sis*-N or with 1 μ g (lanes d, e) or 5 μ g (lanes e and f) of *sis*-C peptide.

human platelet-derived growth factor (PDGF), a potent mitogen for cells of connective tissue origin¹³, have led to the elucidation of its amino terminal amino acid sequence¹⁴. Computer comparison of this and additional protein sequence data with the predicted amino acid sequence of p28^{sis} has revealed an extraordinary degree of homology between PDGF and p28^{sis}, implying that the two proteins have arisen from the same or closely related cellular genes^{15,16}. The present studies were undertaken in an effort to directly establish structural and immunological relationships between the SSV transforming gene product and human PDGF.

Biosynthesis of SSV gene product

The SSV transforming gene codes for a 28,000 MW protein, p28^{sis}. This protein has been identified using antibodies to amino or carboxy terminal peptides predicted from the *v-sis* nucleotide sequence^{11,12}. Biologically active human PDGF is comprised of molecules ranging in MW from 28,000 to 35,000^{13,17-20}. On reduction, PDGF produces biologically inactive, smaller peptides ranging in MW from 12,000 to 18,000^{17,18,21}. By amino-terminal amino acid sequence analysis of both active and inactive forms, two related 18,000 MW peptides (PDGF-1 and PDGF-2) have been shown to be present in PDGF preparations¹⁴. These peptides are around 50% related near their amino termini with no sequence gaps required for homology alignment. The composition of the disulphide linked dimer is not yet known. Recent studies have shown extensive amino acid correspondence of PDGF-2 with a segment of p28^{sis} beginning at position 67 of the predicted 226 amino acid residue polypeptide^{15,16}.

To investigate the structural relationships between the two proteins, we took advantage of observations by some of us (K.C.R. and S.A.A.) that p28^{sis} undergoes cleavage to yield

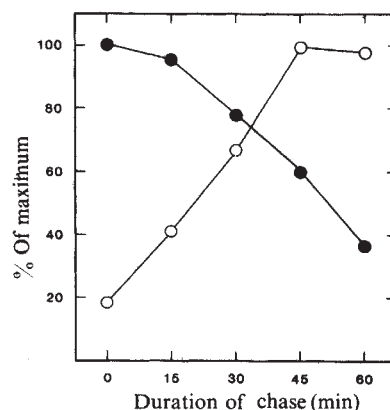


Fig. 2 Pulse-chase analysis of *v-sis* coded proteins. HF/SSV cells, pulse-labelled for 15 min with 35 S-methionine and cysteine, were chased with DMEM supplemented with 250 μ g unlabelled methionine and cysteine per ml for periods of 0, 15, 30, 45 and 60 min. Immediately after each chase, cells were washed in ice-cold phosphate-buffered saline (PBS) and disrupted. Cell lysates were immunoprecipitated with anti-*sis*-C serum and analysed by SDS-PAGE. Fluorographed gels were dried and exposed to Kodak XAR film. Regions of the gel containing p28^{sis} (●) or p20^{sis} (○) were excised using a scalpel. The radioactivity present in each band was determined by liquid scintillation.

two products. As shown in Fig. 1, in addition to p28^{sis}, several lower molecular weight proteins were detected using antibodies prepared against *sis*-N or *sis*-C terminal protides. Proteins of MWs 28,000 and 11,000, which were immunoprecipitated using anti-*sis*-N serum (Fig. 1, lane a), were not detected when the antiserum was preincubated with the *sis*-N peptide (lanes b, c). As a control, preincubation of anti-*sis*-N serum with the *sis*-C peptide had no effect on the immunoprecipitation of these same proteins (lanes d, e). Similarly, immunoprecipitation of p28^{sis} and a protein of MW 20,000 with anti-*sis*-C serum (lane f) was specifically blocked by preincubation of this antiserum with an excess of *sis*-C (lanes i, j), but not the *sis*-N (lanes g, h) peptide. These results indicated that the 11,000 MW protein, designated p11^{sis}, was derived from the amino-terminal region of p28^{sis}, whereas the 20,000 MW protein, designated p20^{sis}, contained its carboxy terminus.

The detection of additional *v-sis* gene products was consistent with the possibility either that these proteins were derived by cleavage of p28^{sis} or were independently initiated translational products. To distinguish between these possibilities, we performed pulse chase experiments. HF/SSV cells were labelled for 15 min, chased for up to 60 min, and analysed by immunoprecipitation. As shown in Fig. 2, the relative intensity of the p28^{sis} band was greatest immediately after the pulse, and diminished thereafter at a rate indicating a half life of approximately 60 min. In contrast, p20^{sis} was observed only after 15 min and increased in intensity for the next 45 min (Fig. 2). Similar results were obtained with p11^{sis} (data not shown). The delayed time of appearance of p20^{sis} and p11^{sis} relative to p28^{sis} strongly suggested that p28^{sis} was the precursor of the smaller two proteins. The N-terminus of the PDGF-2 polypeptide corresponds with position 67 of the 226 amino acid *v-sis*-coded product. Removal of a 66-residue fragment that precedes the section matching PDGF-2 would yield a 160-residue protein with a MW of 18,056, similar to the size observed for the carboxy-terminal cleavage product of p28^{sis}, as well as for PDGF-2.

SSV-transforming protein structure

In view of the known structure of biologically active PDGF²¹⁻²³, we next examined the structure of the *sis*-coded proteins when immunoprecipitates containing these products were processed

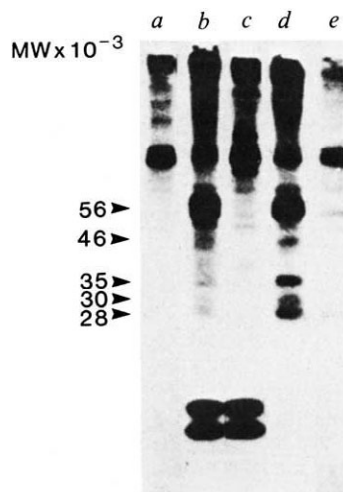


Fig. 3 Analysis of SSV transforming gene products in nonreducing conditions. HF/SSV cells were metabolically labelled as described in the legend for Fig. 1. Cell lysates were immunoprecipitated with preimmune (lane *a*), anti-*sis*-N (lanes *b*, *c*) or anti-*sis*-C (lanes *d*, *e*) serum. In some cases antibodies were incubated before immunoprecipitation with 5 µg of *sis*-N (lane *c*) or *sis*-C (lane *e*) peptide. Immunoprecipitates were analysed by SDS-PAGE in the absence of reducing agent.

under nonreducing conditions. The anti-*sis*-N serum recognized a major species with an apparent MW of 56,000 (Fig. 3, lane *b*). Immunoprecipitation of the 56,000 MW species was specifically blocked by preincubation of the antiserum with *sis*-N peptide (Fig. 3, lane *c*) firmly establishing the *sis*-related nature of this protein. Anti-*sis*-C serum also detected the 56,000 MW protein, as well as four other polypeptides ranging in MW from 28,000 to 46,000 (Fig. 3, lane *d*). Immunoprecipitation of each of these proteins was specifically blocked by preincubation of anti-*sis*-C serum with the *sis*-C peptide (Fig. 3, lane *e*). Our findings that the 56,000 MW protein, designated p56^{sis}, was observed only under nonreducing conditions strongly suggested that it contained disulphide bridges. Moreover, findings that p56^{sis} was detected with antisera directed against both N and C termini of the *v-sis* coded protein further suggested that p56^{sis} represents a dimer of p28^{sis}. The other species, observed only with anti-*sis*-C serum, probably represent processed molecules from which the N terminus of p28^{sis} had been cleaved.

The predicted correspondence of the amino acid sequences of the SSV transforming gene product and PDGF led us to attempt to detect immunological cross reactivity between the two molecules. When immunoprecipitates were made in nonreducing conditions and processed in the presence of a reducing agent, antisera directed against the *sis*-C peptide recognized proteins migrating (after the reduction) with mobilities of p28^{sis} and p20^{sis} (Figs 1, 4). In the same conditions, the anti-PDGF serum detected proteins with electrophoretic mobilities identical to those of p28^{sis} and p20^{sis} from extracts of the SSV-transformed cells (Fig. 4A).

We next reacted extracts of metabolically labelled SSV transformed cells with PDGF antibodies and analysed the resulting immunoprecipitates in nonreducing conditions. As shown in Fig. 4B, all of the nonreduced forms recognized by anti-*sis*-C (lane *b*) serum were immunoprecipitated with anti-PDGF serum (lane *c*). These findings provided strong evidence that the anti-PDGF serum recognized the SSV transforming gene products.

Post-translational processing of p28^{sis} dimer

The presence of multiple molecular weight forms detected both by *sis* peptide antisera and anti-PDGF suggested discrete post-translational processing steps affecting the SSV transforming gene product. The PDGF antibody also detected a protein of MW 24,000 (p24) which did not contain either amino or car-

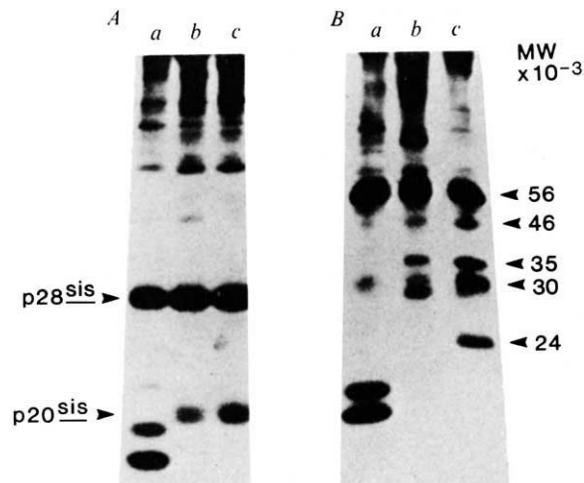


Fig. 4 Anti-PDGF serum recognizes SSV transforming gene products. Lysates of metabolically labelled HF/SSV cells were immunoprecipitated with anti-*sis*-N (lanes *a*), anti-*sis*-C (lanes *b*), or anti-PDGF (lanes *c*) serum, and analysed by SDS-PAGE in the presence (A) or absence (B) of reducing agent. Fluorographed gels were dried and exposed to Kodak XAR film for 16 h.

boxy-terminal regions of p28^{sis}, as judged by its lack of detection by anti-*sis*-N or -C peptide sera (Fig. 4B). In order to examine the time course of processing events involved as well as the derivation of p24, we performed a pulse-chase analysis of the proteins detected in SSV transformed cells with anti-PDGF serum in nonreducing conditions.

As shown in Fig. 5, p28^{sis} was initially detectable but was rapidly converted into the 56,000 MW dimeric form, so that by 30 min only p56^{sis} was present. Subsequently, p56^{sis} was processed in a series of discrete steps to give smaller molecular weight species. The most prominent forms under the conditions of these studies were of MWs 35,000 and 24,000. In other experiments (Fig. 4), 46,000 and 30,000 MW species were clearly detectable as well. None of these species was observed in uninfected marmoset cells (data not shown). Of the forms detected with anti-PDGF, p24 appeared to be the most stable. It was still increasing in amount by 105 min, while all other forms were beginning to decrease in level or were no longer detectable. Its kinetics of appearance as well as lack of detection in uninfected cells established p24^{sis} as a relatively stable processed form of the SSV transforming gene product.

Conclusions

Our present studies directly establish that the SSV transforming gene products and human PDGF bear striking similarities in their structural and immunological properties. We were able to demonstrate that the primary translational product, p28^{sis}, of the SSV transforming gene undergoes rapid cleavage to yield 11,000 and 20,000 MW polypeptides. The latter, which was shown to be derived from the C terminus of the p28^{sis}, is very similar in size to the inactive, reduced 18,000 MW form of human PDGF-2. The sequence correspondence between PDGF-2 and the *v-sis* product begins at position 67. If cleavage of p28^{sis} were signalled by the two basic amino acids (Lys-Arg) at positions 65-66, a 160-residue protein would result, approximating p20^{sis} in its theoretical size. Thus, p20^{sis} closely corresponds in size to that observed for PDGF-2.

Unreduced, biologically active PDGF exhibits a dimeric structure and possesses multiple forms ranging in MW from 28,000 to 35,000^{13,17-20}. The 18,000 MW reduced polypeptide chains, PDGF-1 and PDGF-2, are 50% related near their N termini¹⁴. It is not yet known whether active PDGF is composed of a single protein formed by disulphide linkage of these two peptides



Fig. 5 Pulse-chase analysis of post-translational processing of $p28^{sis}$. HF/SSV cells were pulse-labelled for 15 min with ^{35}S -methionine and cysteine and chased as described in Fig. 2 legend for periods of 0 (lanes a, b), 15 (lanes c, d), 30 (lanes e, f), 45 (lanes g, h), 60 (lanes i, j), 75 (lanes k, l), 90 (lanes m, n), and 105 (lanes o, p) min. Immediately after the chase period, cells were washed in ice-cold PBS and disrupted. Cell lysates were immunoprecipitated with anti-PDGF (lanes a, c, e, g, i, k, and m) or normal rabbit serum (lanes b, d, f, h, j, l and n) in nonreducing conditions and immunoprecipitates were analysed by SDS-PAGE in the absence of reducing agent.

or is composed of two proteins, each of which consists of a disulphide-linked dimer of the homologous peptide. In nonreducing conditions, $p28^{sis}$ possessed a MW of 56,000. Our observations that this polypeptide was detectable with antibodies to both N and C termini of $p28^{sis}$ are most consistent with the possibility that $p56^{sis}$ is a disulphide-linked dimer of $p28^{sis}$. Evidence that the C but not N terminal *sis* antibodies also bound discrete *sis*-related polypeptides ranging in MW from 28,000 to 46,000 implies further processing of $p56^{sis}$ to smaller forms, some of which closely resemble the sizes of unreduced PDGF.

Striking evidence of the close conformational similarity between the two molecules derived from immunological studies. Anti-PDGF serum was shown to recognize the apparent $p28^{sis}$ dimer as well as the other processed unreduced forms of the protein recognized by the anti-*sis*-C peptide serum. In addition,

anti-PDGF recognized a 24,000-MW protein not detected with either anti-*sis* N or C peptide sera. Pulse-chase analysis using anti-PDGF established $p56^{sis}$ as a rapidly formed dimer of $p28^{sis}$. In addition, it was possible to demonstrate further processing in which $p24^{sis}$ emerged as the most stable product of the SSV transforming gene detectable with the available antisera. Our demonstration of cleavage at the N-terminus of the SSV primary translational product, $p28^{sis}$ (Figs 1, 2), implies that the failure of anti-*sis*-N peptide serum to detect processed, nonreduced forms smaller than $p56^{sis}$ is due to cleavage of this moiety from the molecule. Based on its size and lack of detection with anti-*sis*-C peptide serum, $p24^{sis}$ most probably reflects additional proteolytic cleavage at the C-terminus. The dimeric nature of the SSV transforming gene product as well as its rapid proteolytic processing is consistent with the known dimeric structure and susceptibility to proteolysis of PDGF¹⁴⁻¹⁶. Thus, all of these findings suggest that SSV may transform by the constitutive expression of a protein with functions very similar to those of PDGF.

The profound alterations in normal growth regulation induced by the cell-derived transforming genes of retroviruses have many similarities to the growth promoting actions of certain hormones and growth factors. Each exerts pleiotropic effects on cellular metabolism and possesses the ability to induce sustained cell replication. As yet, no other growth factor released either by normal tissues or transformed cells has been shown to be related to the product of any known *onc* gene. However, it is possible that other proteins with growth regulatory functions may be found to correspond to the products of known or as yet uncharacterized *onc* genes.

There have been reports of human osteosarcoma and glioblastoma cell lines which release PDGF-like polypeptides²⁴⁻²⁶. Moreover, *sis*-related transcripts have been detected frequently in human tumours of connective tissue origin, but not in normal fibroblasts or in epithelial cell tumours²⁷. Thus, it is possible that activation of *sis* transcription alone or in combination with other genetic alterations affecting the *sis* structural locus may cause the sustained abnormal proliferation of human cells responsive to the growth stimulatory effects of a PDGF-like molecule. If so, *sis* activation might be implicated as a step in the processes leading normal human cells of certain tissue types towards malignancy.

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Cosmic γ rays and cosmic-ray particles

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Recent experiments using extensive air shower techniques¹⁻⁵ have given evidence for the presence of significant fluxes of cosmic γ rays, in the energy range 10^{15} – 10^{16} eV, from specific cosmic sources. We argue here that the flux from these sources, and others as yet unresolved, is probably sufficient to allow the explanation of a number of previously puzzling features of the cosmic radiation, in particular the way in which the amplitude and phase of the anisotropy of cosmic rays varies with energy. It is possible that the effects of cosmic γ rays will be detectable at energies as high as 10^{17} eV.

Satellite experiments have provided firm evidence for the existence of discrete sources of cosmic γ rays with energies extending into the GeV region, as well as a general diffuse flux attributed to cosmic ray interactions with the nuclei of the interstellar medium (see refs 6 and 7 for recent summaries). The energy spectra of the major galactic sources can be represented by power laws over the energy range of measurement (few tens of MeV to a few GeV) with differential exponents, γ_d , as follows: Crab pulsar, -2.2 ; Vela pulsar, -1.89 ; 'Geminga' (2CG195+04), -1.8 (ref. 8). The energy spectrum of cosmic ray particles (mainly protons) on the other hand is steeper, with a differential exponent of about -2.6 as far as about 3×10^{15} eV and even steeper at higher energies: ≈ -3.1 (Fig. 1a). It is immediately apparent that if the γ -ray exponent (~ -2) continues to much higher energies than several GeV then the γ/p ratio for cosmic rays will increase with increasing energy. It is likely that about 10% of the galactic γ rays at 100 MeV are due to discrete sources, both resolved and unresolved⁹, corresponding to a γ/p ratio of about 3×10^{-6} at this energy (correcting the proton intensity for solar modulation and restricting attention to galactic latitudes within $\pm 10^\circ$ of the galactic plane). The expected ratio at 10^{15} eV, adopting $\gamma_d = -2$ throughout, is thus $\approx 3 \times 10^{-6} \times (10^7)^{0.6}$, that is $\approx 5\%$. Such a high ratio would have important implications for the interpretation of a variety of cosmic-ray phenomena.

The likelihood of an extrapolation of spectra for at least some of the low-energy γ -ray sources being a valid procedure has come from recent direct measurements in the 10^{15} eV region. The first appears to have been the detection of the Crab by Dzikowski *et al.*¹ by way of the observation of muon-poor showers. A measure of confirmation has come from the 'fly's eye' detector² although these authors appear to find variable emission, a feature also found for a threshold of 3×10^{12} eV using Cerenkov detectors¹⁰. (Variability of emission does not materially affect the arguments to be advanced here.) Similar evidence in favour has come from studies of EAS (extensive air showers) at Tien-Shan³ in which EAS poor in both muons and hadrons were observed from the general direction of the Crab (specifically within a circle of 10° diameter centred on this object). The fluxes from all these workers are roughly consistent with $I_\gamma (>10^{15} \text{ eV}) \approx 3 \times 10^{-13} \text{ cm}^{-2} \text{ s}^{-1}$. Coupled with the COS B observation⁸ of $I_\gamma (>10^8 \text{ eV}) = 3.7 \times 10^{-6} \text{ cm}^{-2} \text{ s}^{-1}$ the average differential exponent between these limits is $\gamma_d \approx -2.0$.

A similar situation exists with respect to the well-known X-ray source, Cyg X-3. Recent observations⁴ at Kiel have determined a flux above $2 \times 10^{15} \text{ eV}$ of $7.4 \pm 3.2 \times 10^{-14} \text{ cm}^{-2} \text{ s}^{-1}$, a result based on the detection of EAS over a period of 4 years and one in which the characteristic 4.8-h periodicity is seen. The situation in the 100 MeV region is somewhat obscure, with SAS II finding evidence¹¹ for a 4.8-h signal but COS B not⁸ (although our inspection of the data indicates the probable presence of a signal in some of the observation periods). Combination of the data leads us to estimate a time-averaged flux of $I_\gamma (>10^8 \text{ eV}) \approx 1.0 \times 10^{-6} \text{ cm}^{-2} \text{ s}^{-1}$ and combination with the Kiel flux yields $\gamma_d \approx -2.0$. In fact, the Kiel workers use alternative collections of data, in the hard X-ray region and in the Cerenkov range ($\approx 10^{12} \text{ eV}$), to derive γ_d values of -2.15 and -2.11 .

The Kiel group also present results favouring the detection of five other γ -ray sources with $E_\gamma > 10^{15} \text{ eV}$, albeit of lower statistical significance. Of these, one is probably coincident with the COS B source 2CG135+01, an object that has long been known not to be explainable in terms of an irradiated molecular cloud, at least with the ambient cosmic ray intensity. COS B workers⁸ quote a flux above 10^8 eV of $1.0 \times 10^{-6} \text{ cm}^{-2} \text{ s}^{-1}$ and this, coupled with the Kiel flux, $I_\gamma (>10^{15} \text{ eV}) = (1.5 \pm 0.5) \times 10^{-13} \text{ cm}^{-2} \text{ s}^{-1}$, yields $\gamma_d \approx -2.0$. Six other COS B sources were also examined at high energies but without success and it was concluded that the power law index for these sources was ≤ -2.0 . A comment is necessary here about the six Kiel sources (Cyg

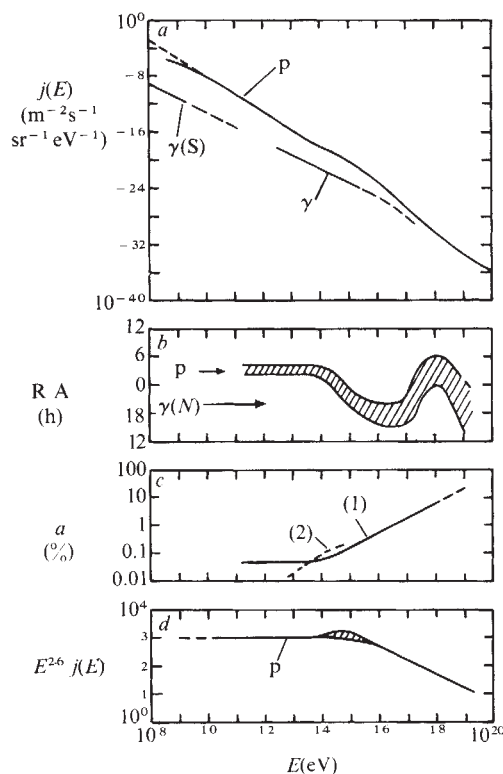


Fig. 1 a, Energy spectrum of cosmic ray particles (mainly protons)—denoted 'p'. $\gamma(S)$: 10% of the average γ -ray intensity with $|b| < 10^\circ$ from satellite experiments⁹. γ : our postulated average γ -ray intensity with $|b| < 10^\circ$. b, Phase of first harmonic of cosmic ray anisotropy for the Northern Hemisphere. 'p' is the postulated phase for protons and $\gamma(N)$ that expected for γ rays. c, Amplitude of first and second harmonics for Northern Hemisphere anisotropies. d, Proton spectrum. Note that the change of slope occurs at a higher energy than the changes in b and c. The shaded area represents a (somewhat contentious) 'bump' in the spectrum.

X-3, 2CG135+01 and four others); it is unlikely that all are genuine (the excesses extend down to the 4.5σ level) but the fact that all are within the latitude range $|b| < 30^\circ$ and half have $|b| < 10^\circ$ gives a measure of confidence that at least several are genuine.

The case has been made for at least some γ -ray sources having spectra which extend out to the 10^{15} eV region, the problem is, how many? A direct answer to this question will not be available for some years but we can proceed by searching for evidence for aggregate γ -ray emission. The standard procedure has been to search for muon-poor showers and there have been claims for a significant number of such showers dating back to 1965¹². The situation is still somewhat obscure, largely because of uncertain fluctuation effects but some recent results from the Akeno EAS array¹³, which relate to energies of order 10^{16} eV, are of interest. The ratio of mean number of muons to number of electrons has been studied as a function of galactic latitude and this is potentially of considerable value. Unfortunately there is a systematic distortion and dispersion but it is interesting to note a dip in the range $b = 0^\circ$ – 10° . Averaging over $|b| < 10^\circ$ (we expect most cosmic γ rays to be in this range) suggests of the order of 1% of the showers as being due to photons, and allowing for the fact that photon showers are probably somewhat bigger than proton showers, in this energy region, we have $\gamma/p \approx 0.5\%$. Such a value is high enough to be encouraging.

Perhaps the most sensitive test of the idea of significant γ -ray fluxes at 10^{15} eV is to examine the anisotropy of cosmic ray arrival directions. After many years of work, consistent results for anisotropies are emerging and there is now a consensus as to the manner in which the amplitude and phase of the first harmonic (the simplest representation of the anisotropy) varies with energy^{14,15}. Figure 1b, c gives a summary of the results. It is certain that below 10^{13} eV the anisotropy relates to the predominant proton component because some of the observations were taken with underground muon detectors which have minimal response to primary γ rays. There are several interesting features, of which the change of phase occurring at about 10^{14} eV, the onset of a rising first harmonic amplitude and the crossing of the second harmonic, all occurring at the same energy ($\approx 10^{14}$ eV), are the most relevant to the present work.

The conventional view is that the change of anisotropy amplitude is related to the onset of increased loss of particles by way of diffusion out of the Galaxy but it can be seen from Fig. 1d that the onset of increased diffusion, as manifested by an increased slope for the proton spectrum, does not occur until about 3×10^{15} eV, a factor of at least 10 higher in energy.

Instead, we postulate that photons are responsible for the change of phase and amplitude, the effect being most marked in the range 10^{15} – 10^{17} eV. An order of magnitude estimate for the intensity of photons can be taken as the proton intensity of protons multiplied by the amplitude of the second harmonic (Fig. 1c) (the geometrical factor due to γ rays being presumably confined to $|b| < 10^\circ$, compared with near isotropy for protons, is disregarded at the present level of treatment). Figure 1a shows the resulting intensity of the differential exponent is $\gamma = -2.2$ and extrapolation back to 10^8 eV gives remarkably good agreement with the intensities of discrete γ rays derived from the satellite experiments referred to above. An exponent of -2.2 is quite reasonable in view of earlier remarks.

Turning to the expected RA of the anisotropy for γ rays, the galactic plane crosses the field of view of most northern detectors at about 6 h and 20 h but we would expect a stronger signal from the latter because of its proximity to the galactic centre ($l \approx 70^\circ$) and 20 h is consistent with observation (Fig. 1b). The second harmonic detected in the range 10^{13} – 10^{14} eV has maxima at 6 h and 18 h (refs 16–18) and this is consistent with the idea of contributions from both protons and γ rays in this range.

There is more evidence of a similar rather imprecise nature which, nevertheless, points in the same direction. Thus, the Chacaltaya experiment¹⁹ in the Southern Hemisphere ($\lambda \approx -16^\circ$) finds a good first harmonic (amplitude $2.9 \pm 1.3\%$) with phase 15.5 ± 1.6 h for 10^{17} – 10^{18} eV, to be compared with an expectation of ≈ 18 h (the galactic plane crosses $\delta = -16^\circ$ at 7 h and 18 h; for the latter $l \approx 15^\circ$ and γ -ray emission will be stronger). At higher energies, 10^{18} – 10^{19} eV, the same group finds a stronger second harmonic, with amplitude $9.0 \pm 5\%$ and maxima at about 7 h and 19 h suggesting that γ rays might be significant here, too, although the Northern Hemisphere data (Fig. 1b) imply that in that hemisphere, at least, protons are predominant.

Inspection of Fig. 1a shows that if the energy spectrum of γ rays were to continue with $\gamma = -2.2$ they would predominate over protons above about 10^{17} eV and this is not allowable, in that many experiments have shown that particles, rather than photons, are responsible for the vast majority (at least 95%) of generated EAS. The γ -ray spectrum must therefore steepen. The origin of this steepening is not expected to be due to galactic propagation effects (although γ - γ interactions with the 2.7-K radiation do have an effect on 10^{15} -eV γ rays, where the interaction length is ~ 8 kpc, and thus have relevance to the Cyg X-3 observations). It is possible that, instead, γ - γ interactions very close to the sources are responsible.

It is premature to speculate on the manner in which the very energetic γ rays are generated but it is interesting to note that, if the Cyg X-3 results are correct and if sources of this type emit 10^{15} eV protons with the same efficiency as 10^{15} -eV γ rays, only about 30 Cyg X-3's are needed in the Galaxy to generate the required intensity of cosmic ray particles.

It is possible that the γ -ray fluxes from the specific sources mentioned (Cyg X-3 and the Crab) have been overestimated and indeed there is the suggestion that this is the case because the numbers of muons detected are higher than expected: they were respectively 77% and 60% of the number expected for primary protons whereas γ rays would have generated some 10% or so. Nevertheless, the showers are deficient in muons and the γ -ray fluxes would appear to be at least 30% of the claimed values. None of the arguments above would be affected.

The conclusion to be drawn is that there are many pieces of evidence, none alone being really significant, but together being coherent, which point to cosmic γ rays of energy above 10^{15} eV comprising a significant fraction (10^{-3} ?) of the primary cosmic radiation.

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Measurements of ^{129}I in meteorites and lunar rock by tandem accelerator mass spectrometry

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The radioisotope ^{129}I (half life, $t_{1/2} = 1.57 \times 10^7$ yr) offers the possibility of extending the time span over which cosmogenic radioisotopes can be used to study extraterrestrial and terrestrial phenomena. Its half life falls between that of ^{53}Mn (3.7×10^6 yr) and ^{40}K (1.28×10^9 yr) in a region where interesting effects can be expected. The target elements for $z > 28$ are normally present in low concentration in natural materials, making detection difficult. We report here the first precise measurements of ^{129}I in extraterrestrial materials: three meteorites of different classes and one lunar surface rock.

The ^{129}I in meteorites is produced by cosmic ray secondary neutron reactions on tellurium¹⁻³, and the ^{129}I in lunar surface materials is produced mainly by spallation on barium and rare earth elements. Because of the difficulty in measuring concentrations of only a few times 10^6 atoms per g in meteorites and about 10 times more in lunar surface materials, only a few preliminary measurements have been reported previously: lunar rock 14321 using neutron activation analysis⁴ and two meteorites by tandem accelerator mass spectrometry⁵. For this study, we selected meteorites with high Te content and large size (more secondary neutrons): Abee (E4), Allende (CV3) and Dhajala (H4). We also selected one lunar rock, 14310, because of its high Ba content and long exposure age.

The ^{129}I measurements were carried out using the University of Rochester MP tandem van de Graaff accelerator as part of an ultrasensitive mass spectrometer. The apparatus was similar to that used in a previous report of ^{129}I measurements⁵. The +5 charge state was selected from the gas stripper at the 5 MV terminal of the tandem. A major improvement was the implementation of a pair of time-of-flight detectors that each use a thin carbon foil at normal incidence to produce electrons which are transported isochronously to a channel plate detector⁶. A timing resolution of 400 ps was obtained with an overall detector efficiency of better than 80%.

About 10 g of ground meteorite were leached with 30 g of NaOH and 15 ml of H_2O after adding 1.05 mg of I^- carrier. After bringing the sample to near dryness the residue was fused with 20 g of Na_2O_2 in a Ni crucible. The melt was extracted with H_2O . The iodine reacted to form an insoluble periodate. The precipitate was separated by centrifuge, dissolved with 1:1 H_2SO_4 and 1 M NaHSO_3 and the solution was acidified to about pH 1. After separation of a small amount of silicic acid precipitate, excess NaHSO_3 was added. The iodide was oxidized to I_2 by 1 M NaNO_2 and extracted into CCl_4 , then back-extracted into 0.1 M H_2SO_4 + NaHSO_3 solution. This step was repeated several times. Finally, the iodide was precipitated as an AgI with ~15 mg of AgCl for increased bulk. The blank and carrier were prepared from brine-well iodine which was obtained from Dr C. T. Pumpelly (Dow Chemical Company). This material originates from salt deposits in the Sylvania sand of

Table 1 Measured ^{129}I concentrations

	Wt (g)	^{129}I (counts)	$^{129}\text{I}/^{127}\text{I}^*$ (10^{-12})	^{129}I atom g^{-1} (10^6)	^{129}I d.p.m. kg^{-1} (10^{-4})
Abee	10.55	293	10.88 ± 0.66	5.10 ± 0.31	4.28 ± 0.26
Allende	10.96	242	6.55 ± 0.43	2.98 ± 0.20	2.50 ± 0.16
Allende	10.36	276	5.98 ± 0.45	2.88 ± 0.22	2.41 ± 0.18
Dhajala	12.15	16	4.17 ± 1.08	1.71 ± 0.44	1.44 ± 0.37
Dhajala	11.45	190	4.30 ± 0.33	1.87 ± 0.14	1.57 ± 0.12
Dhajala	9.68	65	4.23 ± 0.56	2.18 ± 0.29	1.83 ± 0.24
14310,47	1.24	238	8.37 ± 1.12	29.3 ± 3.9	24.6 ± 3.3
Blank (I_2)	—	149	0.13 ± 0.02	—	—

* Data normalized to ^{129}I standard after blank correction.

Table 2 Production rate of ^{129}I

	^{129}I d.p.m. kg^{-1} (10^{-4})	Exposure age (Myr)	^{129}I sat. d.p.m. kg^{-1} (10^{-4})	Te (p.p.m.)	Ba (p.p.m.)	^{129}I (atom min^{-1})
Abee (5,7,0)	4.28 ± 0.26	8.3*	14.0 ± 0.9	2.5§		$0.55 \text{ g}^{-1} \text{ Te}$
Allende (USNM3529)	2.46 ± 0.12	7†	9.3 ± 0.5	1.1	5**	$0.84 \text{ g}^{-1} \text{ Te}$
Dhajala	1.61 ± 0.11	7.5‡	5.7 ± 0.4	~1.3¶	~3.5¶	$0.44 \text{ g}^{-1} \text{ Te}$
14310,47	24.6 ± 3.3	259‡	24.6 ± 3.3	$0.004 \pm$	$\begin{cases} 610^{††} \\ 72^{‡‡} \end{cases}$	$\begin{cases} 0.0027 \text{ g}^{-1} \text{ Ba} \\ 0.011 \text{ g}^{-1} \text{ La} \end{cases}$

* Ref. 7.

† Ref. 8, using new production rate from ref. 10.

‡ Ref. 9; Berdot *et al.*¹⁹ suggested multi-irradiation (see text).

§ Ref. 11.

|| Ref. 12.

¶ Ref. 13.

** Ref. 16.

†† Ref. 20.

‡‡ Ref. 17.

‡‡ La content from ref. 17.

Michigan, of Middle Devonian age. Thus it is at least 20 half lives of ^{129}I old³.

The $^{129}\text{I}/^{127}\text{I}$ ratios and the ^{129}I concentration in the samples are shown in Table 1. The $^{129}\text{I}/^{127}\text{I}$ ratio of the sample was normalized to the National Bureau of Standards standard after a blank correction. The blank had a ratio $^{129}\text{I}/^{127}\text{I}$ less than 1.5×10^{-13} . The background was consistent with that expected from ^{127}I interference; there was no evidence of ^{129}I from cross-contamination or from the original blank material. The errors quoted are derived from scatter in the individual cycles and reflect both statistical and random fluctuations in the ion source output and accelerator transmission. The separate analyses performed for Allende and Dhajala show good reproducibility. A previous result⁵ for the ^{129}I in Dhajala, $1.3 \pm 0.4 \times 10^{-4}$ d.p.m. kg^{-1} , agrees with the present work. Also, the preliminary result⁴ for lunar rock 14321 with 25 Myr exposure age, $(2.1 \pm 1.0) \times 10^{-3}$ d.p.m. kg^{-1} , agrees with the result for the similar rock measured here. The ^{129}I contents have been corrected to saturation levels using the noble gas exposure ages⁷⁻¹⁰ listed in Table 2.

The main target element for production of cosmogenic ^{129}I in meteorites is Te. Kohman² and Lindstrom³ estimated the ^{129}I production rate via $^{130}\text{Te}(n,2n\beta)^{129}\text{I}$ to be 0.40 and 0.70 atom $\text{min}^{-1} \text{ g}^{-1} \text{ Te}$ using the analogous reaction $^{56}\text{Fe}(n,2n)^{55}\text{Fe}$ and measured ^{55}Fe activity in iron meteorites respectively. Using the Te content¹¹⁻¹³, the production rate of ^{129}I from Te can be calculated from the observed ^{129}I content, and is given in the last column in Table 2. The Te content in Dhajala is unknown; thus we used the average Te content in H4 chondrites¹³. These production rates agree reasonably well for the three meteorites, thus we used the average Te content in H4 chondrites¹³. These production rates agree reasonably well for the three meteorites.

The almost 50% difference between Abee and Allende can be explained by considering the ^{129}I production from the $^{128}\text{Te}(n,\gamma)^{129}\text{Te}$ reaction, a possibility not considered earlier¹⁻³. Our Abee sample (number 5,7,0) was located about 13 cm from the surface of a pre-atmospheric body that had a radius of ~30 cm (ref. 14). Allende (USNM 3529) was located about

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30 cm from the surface of a pre-atmospheric body with a 55–100-cm radius¹⁵. The thermal and epithermal neutron contributions should be large for Allende (they produced a very high ⁶⁰Co content, 177 d.p.m. kg⁻¹, in the same fragment¹⁵). We calculated both (n,2n) and (n,γ) production rates of ¹²⁹I at two sample locations using the theoretical production profiles of the analogous reaction of ⁵⁵Fe (Y. Yokoyama, personal communication). The (n,2n) production rates of ¹²⁹I at the two different sample locations are almost the same. However, the (n,γ) contributions are about 10% of the (n,2n) production rate for Abee and 60–70% for Allende. This results in excellent (perhaps fortuitous) agreement for the total measured ¹²⁹I content of the two samples: the production rate of ¹²⁹I by the (n,2n) reaction is estimated to be 0.5 atom min⁻¹ g⁻¹ Te for both meteorites, and the additional ¹²⁹I from the (n,γ) reaction is 0.05 atom min⁻¹ g⁻¹ Te for Abee and 0.34 for Allende.

The Te content in lunar rock 14310 is very low¹⁶. Even though the ¹²⁹I from the cosmic ray-induced fission on U (3.0 p.p.m.)¹⁷ is not negligible (probably ~10% of the total ¹²⁹I)², the ¹²⁹I in lunar surface materials is produced mainly by spallation on Ba and rare earth elements. According to Hohenberg *et al.*¹⁸, the production rates of ¹²⁹Xe are 0.064 atom min⁻¹ g⁻¹ Ba and 0.26 atom min⁻¹ g⁻¹ La (including contributions from other rare earth elements) at the same depth (20 g cm⁻²) as our 14310 sample. We used the chemical composition (610 p.p.m. Ba, 72 p.p.m. La)¹⁷, and the measured ¹²⁹I value, together with the production ratio of ¹²⁹Xe calculated by Hohenberg *et al.*¹⁸, to obtain the fractional isobaric yield of ¹²⁹I out of the total 129 chain for spallation on Ba and La. The result is 4.3%, a reasonable value in terms of spallation systematics. The ¹²⁹I production rates based on this model are given in Table 2. According to Berdot *et al.*¹⁹, the surface exposure age of 14310 is (23 ± 7) × 10⁶ yr. This followed a long exposure time at great depth (≥100 g cm⁻²) in the lunar regolith. If we take into account this multi-stage irradiation history for 14310, the ¹²⁹I production rates in Table 2 will be increased by ~20%.

With the demonstration of measurement capability and consistency of results, this experiment promises that ¹²⁹I may become a powerful new tool for long-lived cosmogenic nuclide studies. We hope to extend these measurements to study the constancy of the cosmic ray flux, meteorite bombardment history, and cosmic ray exposure age dating by the ¹²⁹I–¹²⁹Xe method.

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An unusual interplanetary event: encounter with a comet?

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The interplanetary magnetic field near the ecliptic plane is characterized both by its variability in direction and by its constancy of magnitude. Exceptions to this constancy are depressions in magnitude associated with current sheets and the occasional compressions associated with shocks and stream-stream interactions. Pioneer Venus, orbiting Venus 0.72 AU from the Sun, has encountered an even rarer change in the magnitude of the magnetic field, a slow rise to a sharp maximum and then a decay, surrounding a strong current sheet. Similar structures have apparently not been reported to occur in the interplanetary medium. A survey of three years of both ISEE3 and Pioneer Venus interplanetary magnetic field data reveals what are perhaps small analogues of this feature. However, these features are an order of magnitude shorter in duration and about a factor of three smaller in amplitude. These too are rare, occurring about twice per year on average in both sets of data. An explanation that is consistent with these observations is that Venus passed through the wake of an active comet; other possible explanations seem less likely. However, no known comet was near Venus at the time of this observation. If the observed disturbance was in fact caused by a comet, the symmetry of the signature suggests that it was near perihelion. The signature in plasma and field, described here, indicates that its orbit would be prograde and inclined to the orbit of Venus. The putative comet seems to have approached Venus from above the ecliptic plane.

Figure 1 shows the magnetic field observed by Pioneer Venus over a 24-h period on 10 and 11 February 1982. The data are displayed in solar ecliptic coordinates with the x-axis pointing towards the Sun and the z-axis pointing towards the ecliptic pole. The lighter trace on these plots shows ISEE3 magnetometer measurements at 0.99 AU, 25 h and 20 min later (E. J. Smith, personal communication); this delay is close to the expected transit time for the solar wind which was travelling at 600 km s⁻¹ to reach ISEE3. We have used the times of the major directional discontinuities on the days previous to and following this period to choose the appropriate lag. Venus was 12° past the Earth–Sun line at this time. Thus, the magnetic records at the two locations would be expected to be similar and they are on the time scale of hours both before and after this event. However, during this period there was a slow increase in the magnetic field magnitude, reaching a peak field strength of ~165% of the original field strength. Near the peak in the field strength there was a strong current sheet which was not observed in the ISEE3 data. Then the magnetic field strength slowly declined so that, after about 12 h from the initiation of this event, the magnetic field had returned to its original value.

The source of this event is certainly not Venus itself. The spacecraft is 2.48 Venus radii in front of the planet at the peak of the event, 4.57 radii in the direction opposite planetary

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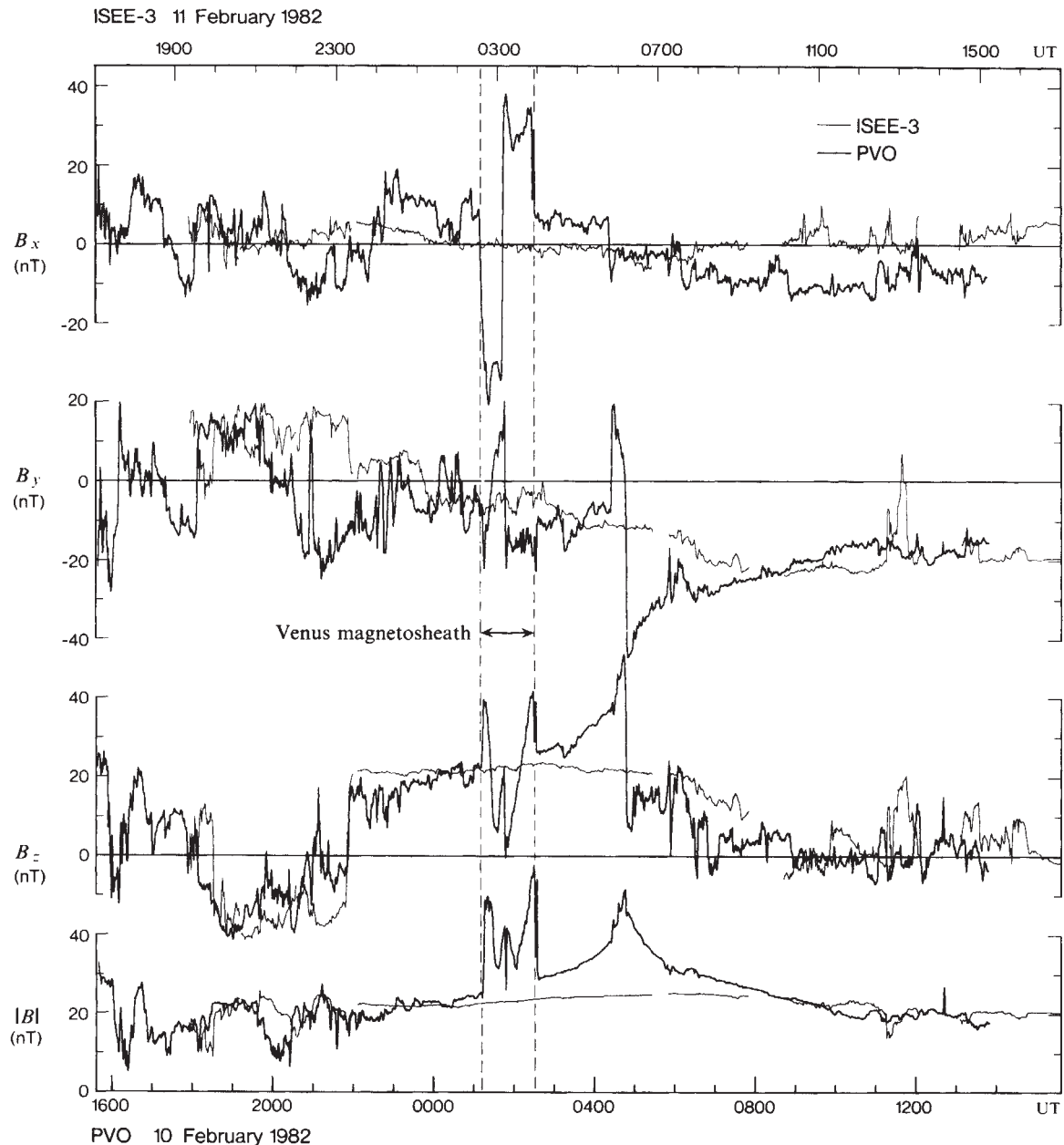


Fig. 1 One-min averages of magnetic field observed by Pioneer Venus (heavy line, PVO) and ISEE3 (light line) in solar ecliptic coordinates. ISEE3 measurements were obtained at 0.99 AU 25 h and 40 min later. Venus and the Earth were 12° apart in the sky as seen from the Sun.

motion and 4.57 radii below the ecliptic plane. No compressional disturbances are expected to be observed upstream of the bow shock in the hypersonic solar wind and never before has such a disturbance been observed. The region enclosed by the Venus bow shock is clearly seen on this plot from about 0100 UT to about 0230 UT. Furthermore, the disturbance is not ordered by its orbital position relative to Venus. It is clearly ordered simply by time. It is a temporal, not a spatial, event relative to Venus.

This event does not seem to be a magnetic cloud¹. Magnetic clouds are long lived, ~ 2 days in duration, with rapid increases in field strength, more constant field strength in the centre of the event, and a gradual rotation of the field direction through the magnetic cloud. The event here slowly rose to a peak that was almost cusp-shaped, and had a sharp rotation in the middle of the event. Furthermore, the event should also have been seen at Earth had it been a cloud as the dimensions of these features are thought to be at least comparable to their solar wind transport scale sizes, that is, ~ 0.5 AU. The longitudinal separation of the Earth and Venus at this time corresponds to 0.2 AU. If this event is a magnetic cloud it is unlike previously reported

examples of this phenomenon. This absence at the Earth suggests, therefore, that the feature is narrower than an interplanetary magnetic cloud. Perhaps we are observing a tail-like feature in the solar wind. Mercury has a magnetic tail of unknown length². Mercury was of the order of 5×10^7 km closer to the Sun than Venus and was far enough from the Venus-Sun line, 24° in heliocentric longitude, for its detection to be highly unlikely even if its tail did extend to 5×10^7 km. Moreover, the magnetic field is not stretched out in a tail-like configuration. The x -component is close to zero, and there is no sudden increase in field strength seen at entry into the event. This event resembles neither the entry into the tail of a planet that has an intrinsic field nor entry into the tail of a planet whose tail is due to the capture of interplanetary magnetic field³.

The signature of this event in the components of the magnetic field is a distortion of the field which peaks at and changes rapidly near the maximum in field strength. We take the undisturbed direction to be the direction of the field detected by ISEE3. This rotation of the magnetic field is very similar to the passage through a planetary magnetosheath. However, we note

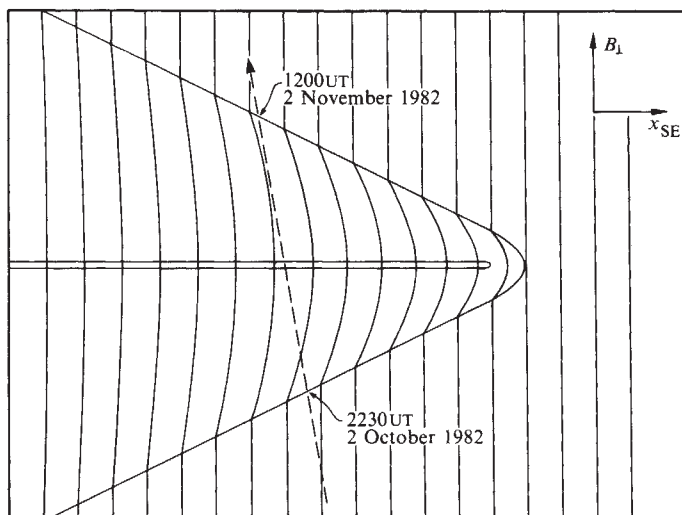


Fig. 2 Interpretive sketch of the observed interaction in the plane containing the solar wind flow and the interplanetary magnetic field but not containing the comet. The trajectory of the spacecraft is believed to have a large component into the plane of the figure and pass close to, but above, the comet tail.

that the disturbance is not bounded by shocks. A comet is expected to be surrounded by an extended disturbance in the solar wind⁴. Furthermore, because of the interaction between the solar wind ions and the cometary neutrals in the extended cometary corona, it has been postulated that there might be no shock in front of a comet⁵. Despite the lack of shocks we shall refer to this region as the possible cometary magnetosheath because of its analogous role to planetary magnetosheaths in the solar wind interaction. As noted above, there seems to be no evidence that the spacecraft entered a magnetotail. The magnetic field was never closely aligned with the planet-Sun line. In fact, it is improbable enough for a comet to pass close to Venus, let alone for the spacecraft to pass through the narrow tail region. Relatively few known periodic comets have perihelia inside of 0.72 AU, and none of these was in a position to cause the observed disturbance (D. Yeomans, personal communication). Nevertheless, the cometary explanation is attractive because of the relative sharpness of the peak of the event. The data suggest a very small obstacle imbedded in a much larger region of interaction.

The measurements of the solar wind probe⁶ and the electron temperature probe⁷ also show behaviour similar to that of a planetary magnetosheath. From 2100 UT to 0430 UT, the density and temperature of the ions both declined about an order of magnitude although the velocity remained roughly constant. After the crossing of the peak in the magnetic field, the density and temperature increased. This is qualitatively the behaviour expected for a traversal of the distant planetary magnetosheath, that is, far behind the terminator⁸. We emphasize that not all the changes in the plasma were gradual. In particular, there was a moderate decrease in temperature and density at 2330 UT on 2 October 1982 and a sudden drop near 0025 UT on 2 November 1982, and a sharp rise in temperature at 1020 UT. In the very centre of the event, some very dramatic changes occurred. At 0437 the temperature, but not the density, suddenly increased to 1.3×10^5 K from $\sim 1.2 \times 10^4$ K. The spectrum at 0447 was too time-aliased to interpret. Then at 0458, the density jumped to over 10 cm^{-3} from $\sim 3 \text{ cm}^{-3}$ previously. The temperature remained high at 1.5×10^5 K.

The helium content of the solar wind is variable due to changes in the location of the solar wind source. We would not expect a comet to be a source of helium. Thus, we examined the time history of the helium content of the flowing plasma to determine whether the magnetic field events from 2230 on 2 October 1982 to 1200 on 2 November 1982 were accompanied by compositional variations. Before this period, Venus had entered a high-

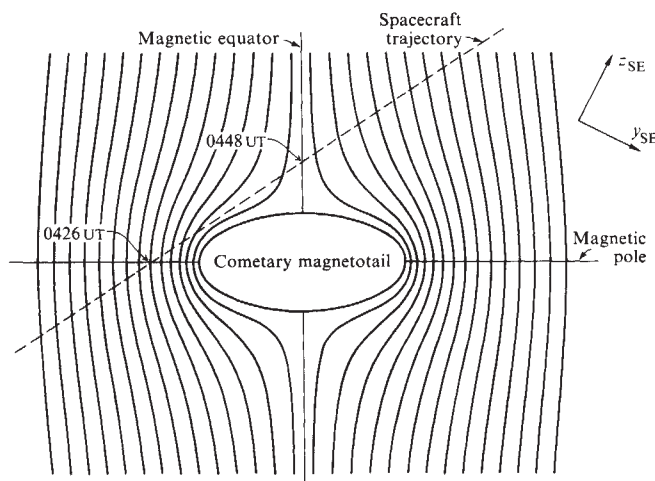


Fig. 3 Interpretive sketch of the trajectory and magnetic field near the cometary tail as the spacecraft passes the magnetic pole and equatorial region. The magnetic equator is defined as the plane containing the cometary tail axis and the external undisturbed magnetic field direction. The magnetic pole is normal to this plane and passes through the tail axis. The coordinate system is comet-centred solar ecliptic.

speed solar wind stream with velocities of up to 700 km s^{-1} . During the period depicted in Fig. 1, the solar wind speed was slowly but steadily decreasing and averaged $\sim 600 \text{ km s}^{-1}$. The helium content was initially quite variable. At about 2000 UT the helium flux exceeded 10% of the proton flux. From 2100 to 2300 UT there were several short spikes of helium enhancement reaching $\sim 10\%$. However, after this period the helium variations settled down and ranged from $\sim 2\%$ to 5% except for a temporary increase to $\sim 8\%$ at 0520 UT. After this time the helium content continued to decline slowly. As these variations did not correlate with the magnetic structure centred around 0446 UT, the helium content suggests that the magnetic structure does not originate from some variations in the source of the solar wind.

The magnetic field was mainly in the positive z solar ecliptic direction throughout the early part of the event both at ISEE3, which did not see the disturbance, and at Venus. If the disturbance was, in fact, a cometary magnetosheath, the sequence of the deviations of B_x , first positive and then negative, indicates that the comet approached Venus from above. This situation is sketched in Fig. 2, showing the draping of field lines in a planetary magnetosheath in a plane some distance to the side of the cometary obstacle. The comet is shown in the background for reference. The plane of the illustration is chosen to contain the solar wind direction and the magnetic field. If the boundary of the disturbed region is really a Mach cone as depicted in Fig. 2, the symmetry of the observed magnetic field profile suggests that the spacecraft (and Venus) passed through the wake at near right angles—in other words, the comet would have been near perihelion. As the x -component of the magnetic field never became dominant during the disturbance, it is unlikely that the spacecraft passed directly into a cometary magnetotail. Furthermore, the magnetic field strength in a cometary tail is not expected to much exceed the interplanetary value⁹. The strength of the field enhancement near the centre of the event, which is comparable to the strength of the field in the strongly shocked Venus magnetosheath, indicates that it probably came close to the obstacle. We note that the ratio of thermal energy to magnetic energy density at ~ 2200 UT on 2 October 1982 was ~ 1.5 assuming an electron temperature of 1.5×10^5 K. If the total pressure was constant throughout this disturbance, only a 60% increase in field strength to 32 nT would occur as the plasma cooled and the density dropped. Thus, this is more than simply a diamagnetic effect in the plasma.

Previously, in discussing the interaction of the solar wind with Venus and the role of the magnetic field in picking up ions¹⁰,

the terms magnetic equator and magnetic pole have been used: the former means the plane containing the solar wind velocity and the magnetic field line that passes through the stagnation point. If, as here, the undisturbed field is nearly vertical, then the equator is nearly vertical. The magnetic poles are the regions on the terminator of the obstacle 90° from the equator over which the field lines must be dragged if they do not penetrate the obstacle. We interpret the field rotations seen in the peak of the event at 0426 and 0448 to be the crossings of the magnetic equator and the magnetic pole of the cometary wake, respectively. Figure 3 shows an interpretation of these crossings in the cometary magnetosheath (we emphasize the qualitative nature of this sketch). The field rotations at the equator and the pole appear to be sharper than this simple picture would predict. Furthermore, the extrapolation of the field in regions away from trajectory rests principally on intuition developed from studies of the terrestrial and Venus magnetosheath and gas dynamic simulations of the same. We do not know of simulations of the solar wind interaction with a comet that could accurately guide our interpretation in this region of space. Note that we have sketched an elliptical cross-section for the cometary tail because this shape will sharpen the rotations in the field. Further, we expect the cometary tail to be elongated in this manner by the Maxwell stresses associated with the ion pick-up process.

Thus, a possible explanation for this disturbance is that an unknown comet passed between Venus and the Sun at this time. If we knew the precise orbit and location of the putative comet, we could use our observations to learn about the dimensions of the interaction region, the amount of mass loading, and so on. As the circular velocity at the orbit of Venus is 35 km s^{-1} , and the escape velocity is 50 km s^{-1} , and as we believe the orbit to be prograde, let us assume that Venus and our object pass at from 10 to 50 km s^{-1} . Then the 12-h duration of this event is equivalent to a distance of $0.5\text{--}2 \times 10^6 \text{ km}$. For a typical solar wind Mach number at Venus of 4.5, the subsolar point of the magnetosheath would be $\sim 10^6 \text{ km}$ to $5 \times 10^6 \text{ km}$ away. We note that, while this disturbance is large on the scale size of the solar wind interaction with Venus, it is small on the scale size of the Venus–Earth separation. Hence, it is expected that this disturbance was not seen at Earth.

After the discovery of this event in the Pioneer Venus records, we searched our data at 0.72 AU and the ISEE3 magnetic records from 0.99 AU for evidence of similar disturbances; no comparable events occurred in seven satellite-years of data. However, events of similar appearance, smaller in both amplitude and duration, were observed. These events had symmetric peaked field disturbances rising 20–80% above background and lasting for 30 min to a couple of hours. It is not obvious whether these are similar events but due to smaller objects, or whether they represent a totally different phenomenon. We intend to examine these smaller features in future studies. Suffice to state here that our observation may not be totally unique and that interplanetary records which have been gathered for many years should be carefully examined for more examples of this phenomenon, which may have escaped detection because of its relative rarity.

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Efficient and stable solar cell by interfacial film formation

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The development of a new efficient and stable semiconductor-liquid junction solar cell is reported. The $n\text{-CuInSe}_2/\text{I}_2\text{-I}^-/\text{Cu}^+/\text{HI/C}$ cell utilizes relatively inexpensive, non-toxic components and requires less stringent sealing which makes this cell a more feasible candidate for energy conversion device applications than its predecessors^{1–3}. Initial output power measurements have already yielded a conversion efficiency of 9.5% under AM1 (Air Mass 1) conditions. After passage of $70,000 \text{ C cm}^{-2}$ the cell continues to deliver virtually unchanged photocurrents at maximum power point. The performance is based on the *in situ* formation of an interfacial film that is stable in the operational range of the solar cell.

Considerable attention has been focused on CuInSe_2 for photovoltaic applications because of its direct bandgap. The low minority carrier diffusion length needed for efficient charge collection is amenable to thin film solar cell construction^{4,5}. Attempts at constructing photoelectrochemical solar cells with ternary chalcopyrites have not yielded cell performances comparable to the existing benchmarks^{6–9}. The better performance of the new cell is attributed to different interface kinetics resulting from the judicious choice of the solution composition and the semiconductor surface preparation.

$n\text{-CuInSe}_2$ single crystals were grown by gradient freeze technique¹⁰ and had a carrier concentration of $2 \times 10^{17} \text{ cm}^{-3}$ and room temperature mobility of $920 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. The crystal was sliced to yield (112) faces, back ohmic contacted with In–Hg, mounted on Cu wires or steel shafts (rotating disk electrodes) with Ag epoxy and encapsulated in Si rubber. The exposed (112) surface was etched in an etching sequence previously reported for CuInSe_2 liquid junctions⁶ consisting of 3:1,

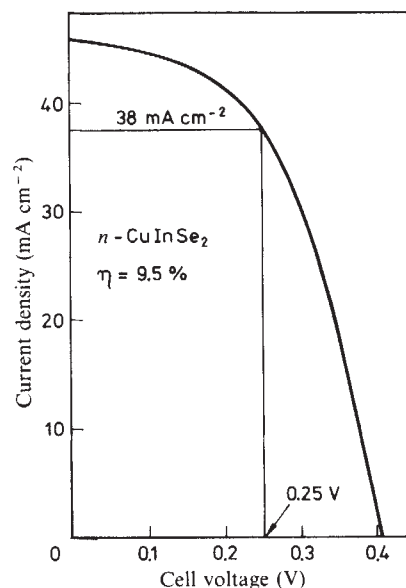


Fig. 1 Output power characteristics of $n\text{-CuInSe}_2(112)/\text{I}_2\text{-I}^-/\text{HI/C}$ cell. Illumination 99 mW cm^{-2} .

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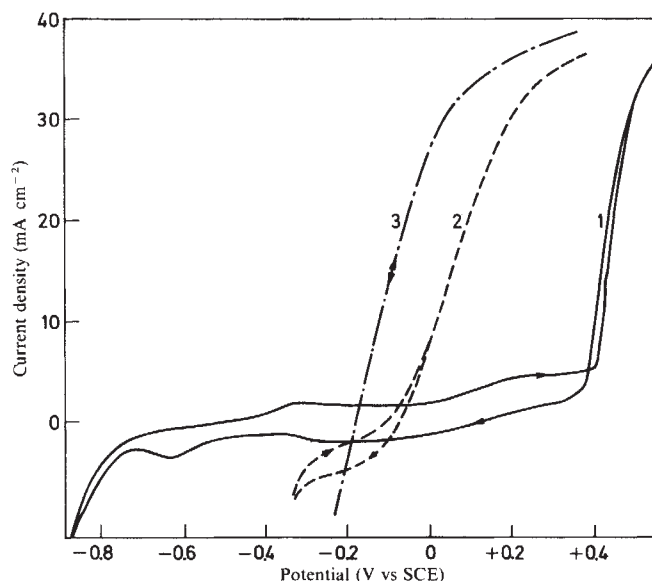


Fig. 2 I - V characteristics of n -CuInSe₂ in 1, 1 M HCl; 2, 1 M CaI₂ + 0.05 M I₂; 3, 1 M HI added to solution 2.

HCl:HNO₃, H₂O rinse, 10% KCN etch, H₂O rinse. A conventional cell provided an optical flat and a three electrode potentiostatic arrangement was used. The electrolyte consisted of 2M I⁻, 2.5 M HI, 50 mM I₂ and 20 mM Cu⁺, and had a potential of 0.16 ± 0.02 V versus SCE. For stability measurements illumination was provided by a tungsten-iodine lamp and for efficiency measurements by natural sunlight or a commercial solar simulator (Oriol).

The performance of CuInSe₂ under AM1 solar or simulated irradiance is depicted in Fig. 1. Maximum power of 9.4 mW cm^{-2} is delivered at 0.25 V and 37.7 mA cm^{-2} yielding an efficiency of 9.5% at 99 mW cm^{-2} incident light. Long-term stability measurements performed near this point correspond to 5 months of steady operation in sunlight and exhibit no measurable alteration in weight nor current output. The stability of the n -CuInSe₂/I⁻-I₂-Cu⁺-HI/C supercedes that of the hitherto known systems although operated in considerably less stringent conditions (solution replenishing, complete isolation and sealing from the atmosphere are unnecessary). By specific control of the reactivity of the semiconductor-electrolyte interface we have been able to arrest the photocorrosion reaction almost completely while retaining a high output power.

Figure 2 shows current (I)-voltage (V) curves at an illuminated n -CuInSe₂ rotating disk electrode at 400 r.p.m. in (1) HCl, (2) I⁻/I₂ redox couple and (3) I⁻/I₂ + HI. With or without illumination curve 1 exhibits a region of low currents which continue to decline on repeated cycling between -0.7 V and +0.4 V versus SCE and may be interpreted as semiconductor passivation^{11,12}. Breakdown of the passivity at more positive potentials > 0.4 V versus SCE, results in photocurrents possibly due to substrate oxidation. A negative shift in the photocurrent onset potential of ~0.6 V is observed in the I⁻/I₂ (curve 2), which locates the maximum power point in the passive region of curve 1. Poor fill factor (FF) and low cathodic currents characterizing curve 2 indicate that passivation continues to dominate the surface kinetics. Addition of HI, curve 3 shows a dramatic improvement in FF, open circuit voltage and short circuit current as well as steeper cathodic slopes. However, the improved characteristics are not permanent.

Surface deterioration is manifested in a gradual decrease of the current output. Addition of Cu⁺ does not alter the I - V characteristics significantly but stabilizes the cell. These results indicate that the interface undergoes specific alterations in this particular electrolyte, sufficiently permanent to sustain high conversion efficiencies over prolonged periods without any sign of deterioration. Thus the stability and efficiency of the cell have

been achieved by adjusting the solution composition, redox potential and pH to maintain an appropriate interfacial film. The effects of the electrolyte constituents on the nature of the film and its relationship to the cell performance are discussed in detail elsewhere¹³.

The new cell presents another advance in construction of efficient and stable photoelectrochemical cells via *in situ* film formation at the interface^{12,14}. It turns out that the manipulation of microscopic interfacial properties with respect to charge transfer, passivation of surface recombination centres and kinetic hindrance of corrosion is of critical importance for the development of any realistic photoelectrochemical device. The design of photoelectrochemical cells with semiconductors exhibiting passivity in the potential range of the respective maximum power point appears particularly promising. On electrochemical surface alteration the kinetics of the interface may then be changed to result in similarly efficient charge transfer as observed in our cell. We consider this criterion equally relevant for selection of semiconductor-electrolyte combinations as the hitherto solely used matching of electron affinity and solution redox potential.

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Viscous magnetization

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The first test of a theory of viscosity has been possible using experimental results published recently by Dunlop¹. This has been accomplished by actually calculating the moment produced as a function of time and temperature. Good agreement is obtained between theory and experiment for all the important parameters which describe viscosity except for the time dependence of the viscosity coefficients. However, the possibility exists that the latter may not be correct. It is also apparent that the average value of the viscosity coefficient is strongly dependent on the grain size distribution. This was predicted theoretically by Walton², but is in disagreement with other theoretical treatments³. It has been possible to explain why the moment acquired in a field of 10 Oe is resistant to demagnetization. This has important implications for experiments which are sensitive to the presence of viscous moments, such as palaeointensity determinations.

Magnetic viscosity is a term first used by Ewing⁶ to describe the time-dependent evolution of the magnetic moment of a sample held in a magnetic field. Since that time it has been a subject of continuing theoretical and experimental interest (see ref. 3 for review). Neel⁷ provided the physical basis for understanding viscosity, and the dependence of the viscous moment on the distribution of grain sizes, and anisotropy constants.

In a more recent theory, since the anisotropy variation from grain to grain is probably much less than the variation in grain size, the anisotropy constant is replaced by a suitable average².

This reduces the expression for the moment to an integral over grain volumes, and it is possible to actually calculate the moment from first principles if the grain size distribution, and other parameters such as the coercive force, H_c , are known.

However, it has not been possible to compare theory and experiment until now. The reason for this lies in the difficulty of performing viscosity experiments. Among other considerations, the change in moment is small, and, as pointed out elsewhere², it depends on the initial state of magnetization. Poor control of this initial state is a feature common to many experimental studies of viscosity.

Dunlop has recently described experiments which are by far the most careful to date. Great care was taken to ensure that the samples were completely demagnetized before beginning a viscous acquisition experiment. In addition, the grain size distribution and the coercive force are available from a previous publication⁸. Thus it is possible to calculate the moment of the smallest grained of Dunlop's samples using the theory in ref. 2 and compare it with Dunlop's results. Unfortunately, the exact concentration of magnetite in the sample is not available (D. J. Dunlop, personal communication), and this must be left as an adjustable parameter.

The multidomain threshold has been calculated by Butler and Bannerjee⁹, and it is clear that the larger grains in the sample exceed this limit. The introduction of a domain boundary must reduce the moment of a grain to a fraction of its single-domain value. Therefore a simple approximation would be to cut off the integration over grain volumes at the multidomain threshold. It is unlikely, however, that the transition from single to multidomain behaviour is determined only by the grain size, the shape and orientation with respect to an applied field should also be important. There is also a nucleation problem¹⁰. This would lead to a transition region in which the effective moment would decrease as the grain volume increased. No attempt was made to include this effect in the treatment to be presented here, since this would require the introduction of additional parameters. Thus, the effect of average grain size on magnetic viscosity will not be addressed, although this is one of the major considerations of Dunlop's paper. However, it is worth observing that a sharp decrease in the viscosity coefficient occurs in the neighbourhood of the single-domain threshold, which is in agreement with the qualitative argument presented above.

From ref. 2, the moment produced in an initially demagnetized sample in a field H , after a time t , is given by

$$M = \int J V n(V) N(V) dV \quad (1)$$

where V is the volume of a grain, J is its saturation magnetization, $N(V) dV$ is the number of grains between V and $V + dV$, and $n(V)$ is the fractional alignment.

The fractional alignment, $n(V)$, is time dependent and may be calculated as follows, assuming that the rotation of the moment is thermally activated:

$$dn/dt = (n - L(a))w \quad (2)$$

where t is the time, $a = JHV/kT$, $L(a)$ is the Langevin function which is the equilibrium fractional alignment, and w is a rate given by

$$w = c \exp(-KV/kT)$$

where T is the temperature, K is the anisotropy constant, which is also temperature dependent, k is Boltzmann's constant, c is the fundamental frequency for rotation of the moment of the grain, c is on the order of 10^8 s^{-1} . Integration of equation (2) yields n :

$$n = L(a) + (n_i - L(a)) \exp(-wt)$$

where n_i is the value of $n(V)$ at $t = 0$.

Equation (1) now yields the sample moment:

$$M = \int J V [L(a) + (n_i - L(a)) \exp(-wt)] N(V) dV \quad (3)$$

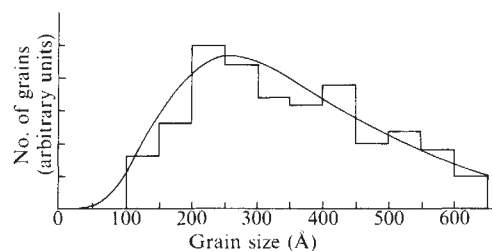


Fig. 1 The grain-size distribution determined from electron microscope observations, taken from Dunlop, fitted to the log-normal distribution shown. Equation for curve: $x = 1/r \exp(-\frac{1}{2}[(\ln r/350)/0.55]^2)$.

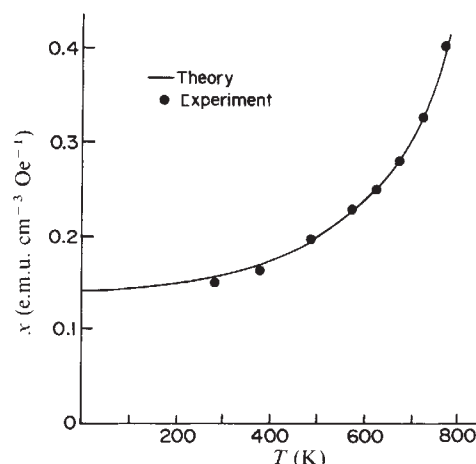


Fig. 2 Comparison between calculated and measured susceptibilities as a function of temperature. The susceptibility is the magnetization at the end of 5 s divided by the field.

Actually, the anisotropy will not be the same for all the grains so in principle an integration should also be performed over K . However, for magnetite K is determined by the shape of the grain. If it is assumed that they are of similar shapes K should be constant. This should, in fact, be a good assumption for Dunlop's sample since⁸ electron microscope examination reveals that the grains are cubic. If H is large, it is necessary to take into account the effect of the field in lowering the barrier to rotation of the moment. Using the expression for the relaxation time given by Nagata¹¹, and neglecting terms in $(H/H_c)^2$ the frequency factor c in the expression for w becomes $c \cosh(a)$.

The magnetic particles were produced by precipitation, and it is commonly agreed¹² that the size of the particles produced in this way follows a log-normal distribution. Therefore a log-normal distribution was chosen that yielded the average size, and variance measured. Figure 1 shows this distribution plotted against the size distribution obtained using the electron microscope. Actually it is possible to use this observed distribution in equation 1, but the calculated moment is extremely sensitive to $N(V)$, and the discontinuities in the experimental size data lead to large, and probably unphysical, fluctuations.

Equation 3 was then integrated numerically using the following values:

The initial values of n , $n_i = 0$ for acquisition. J was obtained from values published by Pauthenet¹³, K was obtained using the equation¹¹, $K = JH_c/2$ and the values of H_c published by Dunlop⁸. This equation is derived for elongated particles, and, since the particles in Dunlop's samples are cubic, a different value for the denominator is appropriate. However, the calculation of the demagnetizing factor is quite difficult, by default, then, equation 2 was used.

The moment obtained by integration of equation 3 was added to the instantaneous moment produced on application of the field, the IRM. The latter was not calculated, but was obtained

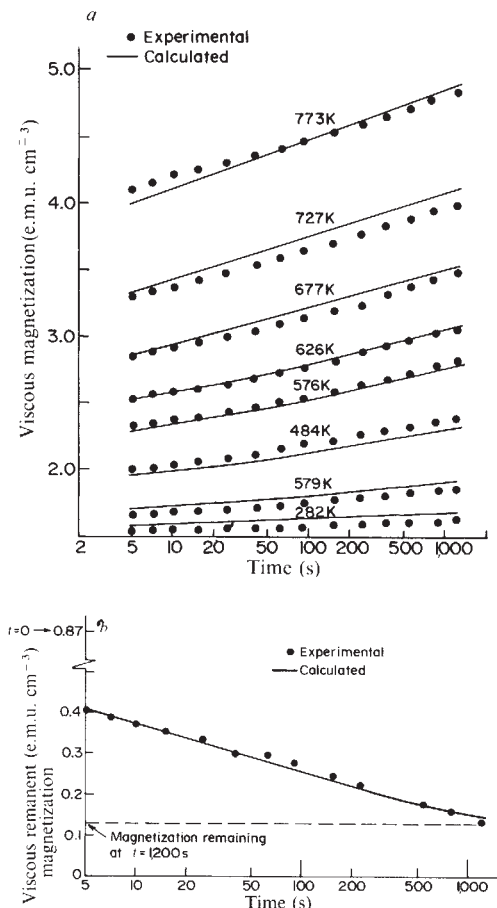


Fig. 3 *a*, Comparison between calculated and observed magnetization as a function of time and temperature. The only adjustable parameter utilized was the magnetic concentration, thus the slopes and relative positions of the solid lines are established by the theory. *b*, Comparison between the calculated and observed decay of the viscous remanent magnetization at 484 K. Since the initial values were not available the calculated line, unlike Figs 3*a* and 2, has been arbitrarily positioned to intersect the experimental points. This result shows that a substantial fraction of the initial moment remains after demagnetizing for the same length of time as that for which the sample had been magnetized. This appears to be a result of the relatively large magnetizing field, 10 Oe. Calculation indicates that a smaller field of 1 Oe would result in only about 5% of the moment remaining at the end of a similar experiment.

by extrapolating the susceptibility to zero temperature, and multiplying by the field.

It is also possible to calculate the susceptibility. The result of this calculation is shown in Fig. 2, and compared with the experimental values which are equal to the magnetization produced after 5 seconds had elapsed divided by the field.

The results of calculating the moment acquired in a 10 Oe applied field as a function of time and temperature are shown in Fig. 3*a*. This result shows that equation 1 accounts satisfactorily for both the time and the temperature dependence of the magnetization.

The decay of the moment in zero field was also calculated as follows:

The initial value of n is its value at the end of the moment acquisition run:

$$n_i = L(a)[1 - \exp(-wt_2)]$$

the value of n at equilibrium is, of course, zero. Thus

$$n = L(a)[1 - \exp(-wt_2)] \exp(-wt)$$

It should be noted that the field is now zero, hence the factor $\cosh(a)$ no longer effects the relaxation time.

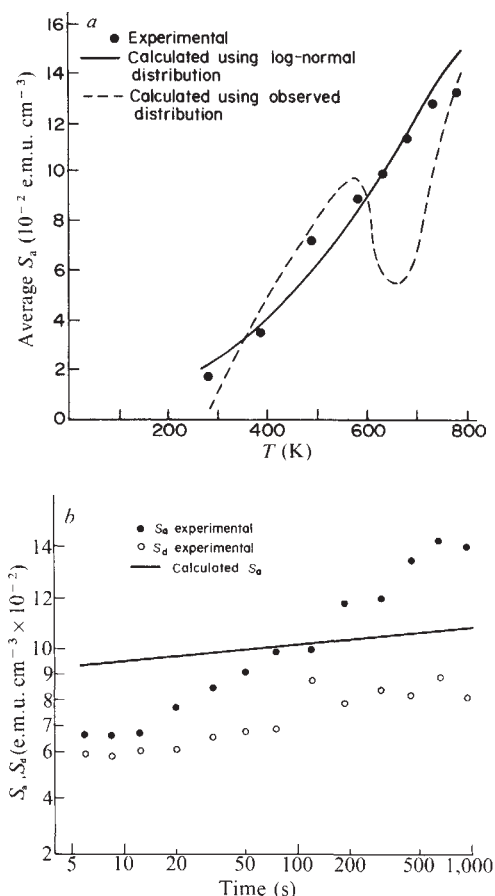


Fig. 4 *a*, Comparison between calculated and observed average viscosity coefficients. The solid line was calculated using the log-normal distribution from Fig. 1, the dashed line using the histogram of the observed grain sizes⁸. *b*, Comparison between calculated and observed time dependence of the viscous acquisition and decay coefficients. Although the theory predicts a linear increase with $\ln t$, the slope of the experimental acquisition curve cannot be reproduced theoretically unless an anomalously low value is used for c .

These values were substituted in equation 1. Numerical integration then yielded the result shown in Fig. 3*b*, results are only presented for one temperature because the initial values of the magnetization were unavailable, thus the displacement of the curves with temperature could not be compared with experiment. Figure 3*b* shows the decay at 484 K, and it is clear that a substantial fraction of the initial moment is still present at the end of the 20 minute period, in this case 16%. This was also the case for the other temperatures.

Dunlop comments extensively on the difficulty of removing the viscous moment acquired by these samples. The explanation is provided above, and is the result of the reduction in the relaxation time produced by the applied field.

If the field were to be reduced to 1 Oe the moment remaining at the end of the decay is calculated to be 5%. The reason it should not be removed completely lies in the fact that the initial distribution has a tail that extends to large grain volumes. This result has important implications for experiments which are sensitive to the presence of viscous moments.

The next important parameter is the viscosity coefficient, $S = dM/d \ln t$. The average value of S was calculated by taking the difference between the moment at 20 min and that at 5 s, and dividing by the natural logarithm of 240. The results are shown in Fig. 4*a*, and while the agreement is satisfactory, the discrepancies are significant. The increase in S between 379 K and 484 K is repeated in the experimental demagnetization data, and indeed for other concentrations. This is probably the result of fluctuations in $N(V)$. S is very sensitive to $N(V)$, this is illustrated in Fig. 4*a* by the result of calculating S using the

published grain size distribution⁸, which is given as a histogram. The steps in grain size are largely responsible for the large changes in the calculated values. Nevertheless an increase in the neighbourhood of 500 K is present, which suggests that the 'hump' in that region is due to a 'hump' in $N(V)$, since there is a suggestion of a secondary maximum in the published histogram.

It is easy to understand why S should be sensitive to $N(V)$: if equation 3 is differentiated with respect to t the integrand in the resultant integral is only significant near $V = (kT \ln ct)/K$, thus S probes $N(V)$ in the neighbourhood of this value. In fact this restricted dependence of S on V was used in ref. 2 to calculate S , and obtain an analytical expression for M .

While it was possible to account satisfactorily for the temperature dependence of the average value of S during acquisition, it has not been possible to do this for the time dependence. One of the main predictions of the theory² was that S should be proportional to $\ln ct$ for $N(V)$ proportional to $1/V$, which is approximately true for a log-normal distribution. This is

indeed the case for both the calculated and observed results, the problem lies in the magnitude of the slope. This is illustrated for a temperature of 484 K in Fig. 4b.

A clue to the reason for the difficulty can be obtained from the experimental data: if the plots of S against $\log t$ in Fig. 5 of ref. 1 are extrapolated it will be found that S becomes 0 at t on the order of 10^{-3} . Thus $c = 10^3$, an unusually low value. If the procedure is repeated for the decay coefficients a value of c around 10^6 is obtained, which is more reasonable.

On the other hand, M must also be 0 at times which are less than $1/c$. Linear extrapolation of experimental values of M to 0 in Fig. 3 indicates that this occurs at a t of about a microsecond. Since M is actually not linear in $\ln ct$ this must be an overestimate. Thus it appears that the two sets of experimental results are not consistent, and this point requires further investigation.

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A stress province boundary and tractions on the North American plate

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Stress provinces, characterized by nearly constant directions and relative magnitudes of horizontal principal stresses, are potentially informative of the forces acting on a lithosphere plate. This applies most strongly to stress provinces which occupy a large part of a plate, such as the Mid-Continent Stress Province of North America identified by Zoback and Zoback¹. We have used fractures known as breakouts in oil-wells, which are controlled by the *in situ* stress, to show that this stress province extends through the western Canadian sedimentary basin to the arctic coast. There is a strong possibility that a uniform stress orientation, with greatest compression NE–SW, is co-extensive with the North American continent east of the Rocky Mountains excluding the Appalachian and Gulf of Mexico stress provinces. We further report new breakout orientations in the far northwest of Canada, which locate the boundary between the Mid-Continent Stress Province and an Alaskan stress province, with largest compression NNW–SSE, probably controlled by the subduction of the Pacific plate under Alaska.

The orientation of the stress tensor in the Earth's crust within a tectonic plate largely reflects the forces acting on that plate. At depths of 10 km or less one principal stress is usually nearly normal to the Earth's free surface and so nearly vertical. The magnitude of this vertical principal stress at depth z tends to lie within about 20% of the 'lithostatic stress' $\rho_r gz$, where ρ_r is the integrated density of the overlying rock². In general the horizontal principal stresses are unequal and in regions of compressional tectonics one of them exceeds the vertical principal stress^{2,3}. For plate tectonics special interest attaches to the orientation and magnitudes of the two horizontal principal

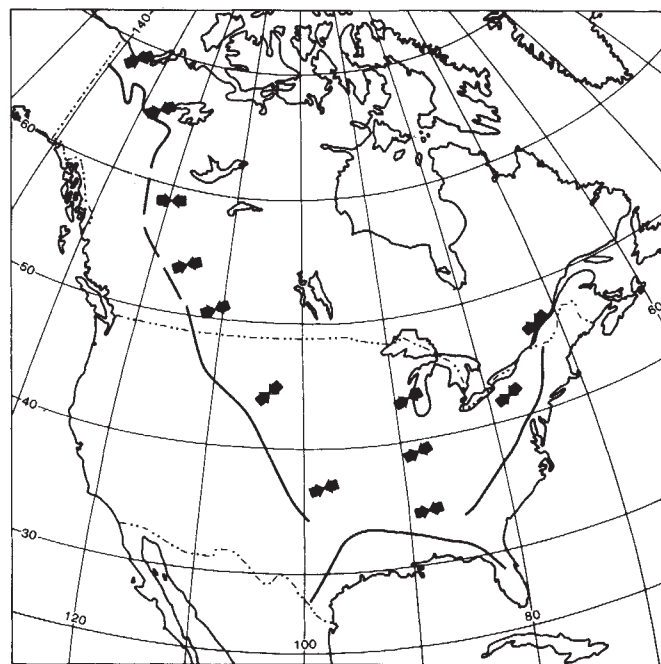


Fig. 1 The Mid-Continent Stress Province of North America. The boundary is drawn from Zoback and Zoback¹, extended in western Canada from our work on breakouts in oil-wells⁹. The pairs of broad arrows give a generalized representation of the direction of the greater horizontal principal stress, S_H . Each pair of arrows represents between 3 and 30 stress orientation data.

stresses. There are several possible causes of stress within a plate, including various tractions and body forces³. Although the interpretation of intraplate stress data in terms of tractions on the plate must be approached with other possible causes in mind, the orientations of intraplate stress fields contain important information on plate driving forces^{1,4}. Considerable work is now in progress on crustal stresses, through overcoring strain relief techniques, earthquake source mechanisms and hydraulic fracture experiments⁵.

The relevance of stress orientations to tractions on a plate is clearest when those orientations are coherent over a large part of the plate. Zoback and Zoback¹ have used stress measure-

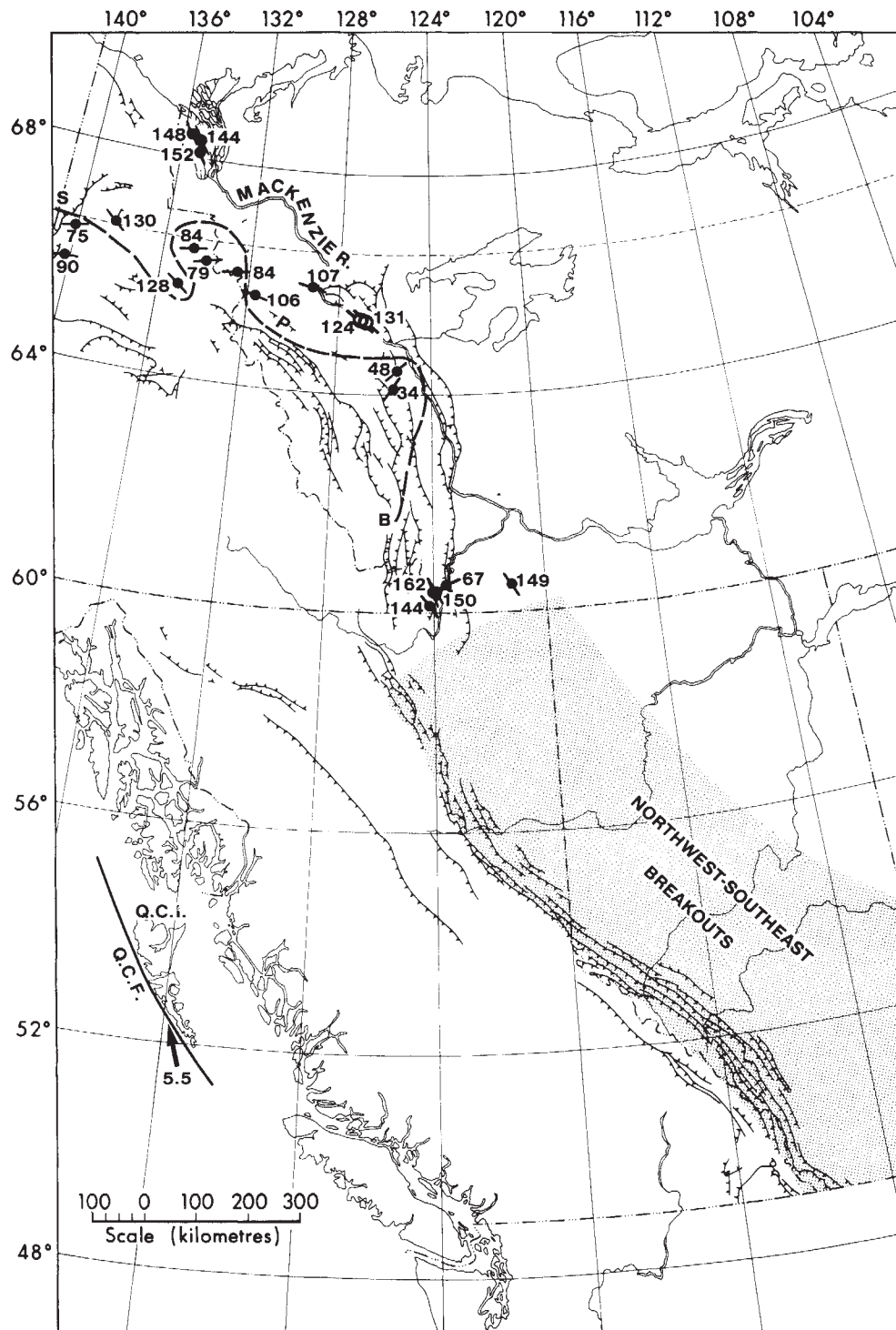


Fig. 2 Breakout orientations in 21 oil-wells in northwestern Canada. The mean direction of maximum elongation (and of the lesser horizontal principal stress, S_H , on our hypothesis^{14,15}) is indicated for each well by a bar and a number which gives the azimuth in degrees east of north. SPB is a proposed stress province boundary. The major thrust faults are shown, as are the Queen Charlotte Islands (QCI) and Fault (QCF).

ments, hydraulic fracture results, earthquake mechanism solutions, geological data on young fault displacements and volcanic alignments to delineate 14 stress provinces in the conterminous United States. Of these one, the Mid-Continent Stress Province, is largest and extends from the east front of the Rocky Mountains of Colorado and Wyoming to New England and the Atlantic provinces of Canada. This extensive stress province is characterized by a NE-SW direction of the greater horizontal principal stress S_H , which is often larger than the vertical stress^{1,6-8}. We have recently shown⁹, from a class of fractures known as breakouts^{10,11} in the walls of oil-wells, that the coherent NE-SW orientation of S_H extends throughout the western Canadian sedimentary basin, northeast of the Rocky Mountains of Alberta and British Columbia. Figure 1 shows the directions of S_H where these are supported by the stress orientations^{1,9,12} already cited, in the Mid-Continent Stress Province of Zoback and Zoback as

extended by our work in western Canada. NE-SW alignments of S_H surround the Canadian Shield on three sides and very possibly extend through the shield in a coherent stress field of the North American craton. Zoback and Zoback¹ and McGarr¹³ have suggested that the stress field may indicate northeastward basal drag on the plate, and relate this to southwestward motion of the plate over a passive asthenosphere. Alternatively large-scale mantle convection could be driving the asthenosphere and the plate north-eastwards, to produce the same northeastward drag on the base of the plate⁹.

We have now examined breakouts in 19 oil-wells in the Yukon and Northwest territories of northwestern Canada. Breakouts elongate the cross-section of a vertical hole in rock under unequal horizontal principal stresses, as a result of shear fracturing in the amplified stress difference near the hole caused by the hole itself^{14,15}. The resulting elongations are found to be

closely grouped in azimuth both within a given well, and between wells in a province of constant stress orientation, with the long axes indicating the orientation of the lesser horizontal principal stress S_h . The long axes of breakouts in the western Canadian basin (shaded in Fig. 2) are concentrated around a NW–SE direction, implying that S_H is directed NE–SW. It has been found that break-outs in three stress provinces in the United States and one in Canada give S_h directions in agreement with other, independent stress tensor data^{9,12,15}.

Commercially available four-arm dipmeter logs provide the breakout data. We began our study of the logs from the 19 wells in the far northwest of Canada, in the expectation that the stress field of the western Canadian basin would be found to extend there. In Fig. 2 the mean azimuths of breakout long axes are shown for these wells and for two others, at Norman Wells on the Mackenzie River, previously reported¹⁵. The number beside each well location is the mean azimuth of the long axis of the breakouts in that well in degrees east of north. On our hypothesis^{14,15} this is also the direction of S_h . For comparable data in Alberta the standard error of the mean azimuth from a well is typically less than 5 degrees⁹. Breakouts with azimuths between 106° and 162° are observed in 13 wells covering the latitude range 60° to 68.5° N. These results extend the stress orientation characteristic of the craton of North America to the arctic coast at the Mackenzie Delta. The other 8 wells yield breakout azimuths in the range 34° to 90°. Seven of these oil-wells lie south and west of the line SPB shown in Fig. 2. We consider this line to represent part of a stress province boundary between the Mid-Continent Stress Province and an Alaskan stress province in which S_H is approximately NNW–SSE as a result of traction on this part of the North American plate associated with subduction of the Pacific plate along the Aleutian island arc. There is abundant evidence, from earthquake mechanisms^{16,17}, from the azimuthal distribution of volcanic vents and geological data⁴, of a NW–NNW orientation of S_H in the Aleutian arc and Alaska, parallel to the generally accepted direction of subduction of the Pacific plate¹⁸. Some stress orientations from breakouts in wells off the south coast of Alaska¹⁹ agree with the other indicators in showing NW orientation of S_H .

Note that the proposed stress province boundary is well within the North American plate and about 800 km from the downturn of the subducted Pacific plate. The boundary is also well within the craton; it lies about 500 km north of the Denali Fault which marks the edge of the craton against the microcontinent assemblage now known as Wrangellia. The strikes of the major thrust faults shown in Fig. 2 rotate from NNW in the Mackenzie Valley to roughly E–W as the Alaska/Yukon border is approached. In some places the breakout azimuths are roughly parallel to the thrust faults, though it would be difficult to quantify that statement. This suggests that at least some of the displacements on the thrusts may have occurred in a stress field oriented like that now in existence. Geological evidence of young offsets would be of great interest but we have no knowledge of such data for the region.

Several minor points of interest in Fig. 2 deserve mention. At 60.5° N, 123.6° W a well shows an 'Alaskan' orientation of S_h (on our hypothesis) among four wells showing a Mid-Continent Stress Province orientation. Mixed orientations near this location have been reported elsewhere^{9,12} and there may be small regions here carrying the stress fields of the two stress provinces. In principle this could arise through discontinuities in coupling of the rock in the sedimentary basin to the basement and to the plate. Alternatively S_H may be nearly equal to S_h in this locality. We have not attempted to map a highly convoluted boundary here because present information would not justify this. Earthquakes in the region are small and have not yielded mechanisms. They are concentrated near the stress province boundary so as to indicate appreciable tectonic activity there²⁰.

The Queen Charlotte Islands (QCI) and Fault (QCF) are shown in Fig. 2. It has recently been shown²¹ that the Queen Charlotte Fault accommodates some oblique underthrusting of

the Pacific plate in addition to the mainly transform-fault relative motion. The arrow in Fig. 2 shows the velocity vector of the Pacific plate relative to the North American plate, with a magnitude of 5.5 cm yr⁻¹ (ref. 21). The velocity vector has a direction close to that of S_H indicated by the breakouts in the five wells west of 132° W and south of the line SPB. Both are consistent with the azimuths of maximum compression in Alaska indicated by volcanic vents⁴.

An intensive forthcoming study of breakout orientations in Alaska (K. H. Jacob, personal communication) should greatly improve both the areal coverage and the precision of knowledge of the location and width of this boundary between the two stress provinces. It is to be hoped that study of four-arm dipmeter logs will soon extend understanding of stress orientations in many sedimentary basins undergoing exploration for hydrocarbons, and ultimately of the forces that drive the plates.

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Why large earthquakes do not nucleate at shallow depths

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Although by definition tectonic earthquakes may nucleate anywhere within the seismogenic layer, in almost all cases those earthquakes that become large nucleate near the base of the seismogenic layer. Because frictional strength and stress-drop should increase with depth and *in situ* stress measurements suggest that the ambient stress increases with depth, we have examined spontaneous rupture models with gradients of both average stress-drop and average strength relative to ambient stress to determine whether this explains the observed phenomena. We report here that ruptures that are nucleated within the low stress-drop (shallow) regions of the model are inhibited from propagating, but those that are nucleated within the high stress-drop regions can propagate over the entire fault plane.

Although small earthquakes nucleate over a broad depth range within the seismogenic layer, a prominent feature of very large earthquakes is that they almost invariably nucleate near the base of the seismogenic layer. This observation is almost always true for large earthquakes for which reliable hypocentral depths and rupture dimensions are available. It is also borne out for great subduction zone thrust events for which the main-shock epicentre is almost always found to lie on the extreme landward, and hence deepest, part of the aftershock zone¹. In

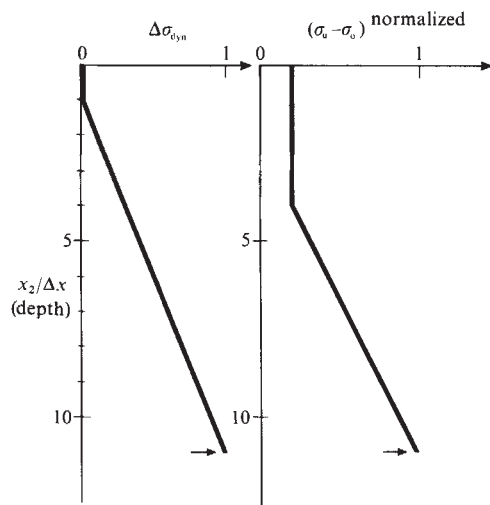


Fig. 1 The normalized dynamic stress-drop ($\Delta\sigma_{\text{dyn}}$) and the critical stress for fracture ($\sigma_u - \sigma_o$) as a function of ($x_2/\Delta x$) in an infinite, homogeneous, elastic medium. These parameters are plotted only in that region of the whole space influencing the calculations.

the western United States, the larger earthquakes tend to originate near the base of the seismogenic layer². As in principle any earthquake, once nucleated, has the potential to propagate large distances, these observations indicate that this potential is strongly biased in favour of those events that nucleate at the greatest depth.

Laboratory studies³ show that the frictional strength of a fault increases with normal stress. As the normal stress across a fault increases with depth, the frictional strength of the fault should increase with depth in the seismogenic layer. Such studies also show that the stress-drop during stick-slip is proportional to the frictional strength^{4,5}, which suggests that the stress-drop should also increase with depth. There is some recent evidence indicating this for small earthquakes at Oroville, California⁶ and at New Brunswick, Canada (J. Boatwright, personal communication) but further systematic study is needed to confirm this.

The frictional strength and stress-drop are two of the three parameters that control rupture growth, the third being the ambient applied shear stress. It seems reasonable to assume that at the time of a large earthquake the applied stress is close to the frictional strength throughout so that it also increases with depth. This is supported by *in situ* stress measurements in California⁷ which extended to a depth of 1 km. Our assumption extrapolates this linear increase in stress to the base of the seismogenic layer. For simplicity, we assume that at the time of a large earthquake the applied stress is a constant fraction of the frictional strength at all depths.

Based on this notion, we consider a numerical model of crack propagation in an infinite, homogeneous, elastic medium where the dynamic stress-drop⁸ and the critical stress (or equivalently, the frictional strength), relative to the ambient stress, required for slip increase with depth. (The different types of stresses and stress-drops are defined in ref. 8.) The dynamic stress-drop, $\Delta\sigma_{\text{dyn}}$, and the excess of the critical stress σ_u over the ambient stress σ_o , both normalized by the value of the stress-drop at the depth indicated by the arrows, are plotted in Fig. 1. The critical stress in the upper part of the faulting region is not allowed to reach zero but is instead constrained to be small. As there is evidence of a gradient of frictional strength relative to the ambient stress and of stress-drop with depth as discussed above, we have arbitrarily selected such gradients to determine whether the gradients alone can explain the phenomenon that we are exploring. Clearly, if these gradients are zero, no such selectivity on the depths of hypocentres of large earthquakes will be predicted. Due to the arbitrariness of choice of gradients, we shall draw conclusions which are purely qualitative.

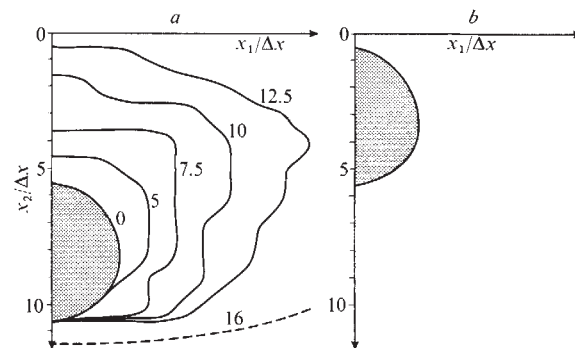


Fig. 2 Fracture front at successive normalized times on the ($x_1 - x_2$) plane, which is the fault plane, for the two cases studied. *a*, Crack nucleated at the higher stress-drop, deeper region of the seismogenic layer and dynamically propagates into a large earthquake. *b*, Crack nucleated near upper, low stress-drop region of seismogenic layer and cannot propagate due to lack of sufficient strain energy.

We assume that a circular crack suddenly appears and starts radiating. The nucleation and growth of the fracture are solved using the numerical boundary integral technique⁸ and a 'critical stress level' fracture criterion, in which a grid point ahead of the crack edge breaks when the stress averaged over the grid exceeds a critical level⁹. The crack is allowed to propagate over an infinite plane in an infinite elastic medium and there are no artificial barriers to prevent propagation anywhere on the fault plane. The parameter that actually controls our results is in fact the ratio of ($\sigma_u - \sigma_o$) to $\Delta\sigma_{\text{dyn}}$.

We have studied two cases. In the first, we allow the initial crack (indicated by the stippled region in Fig. 2*a*) to nucleate in the lower part of the seismogenic region. The fracture is allowed to propagate spontaneously and the fracture front as a function of normalized time $at/\Delta x$ (where a is the compressional wave speed of the medium and Δx is the spatial grid size in the numerical scheme) is shown on the fault plane, which is the ($x_1 - x_2$) plane, in Fig. 2*a*. Only one half of the fractured area is plotted, the other half being a mirror reflection of this about the x_2 axis. Clearly, the crack can develop into a large earthquake. At the normalized time 12.5, the crack had propagated only upwards and laterally. To check whether any propagation in the downward direction occurs at a later time, the calculations were continued for a longer time. It was found that by normalized time 16.0, the crack had propagated only a small distance downwards (dotted line in Fig. 2*a*). This means that the crack propagation velocity in the downward, higher strength, direction is much smaller than that into upper, weaker, regions. This last effect was also seen by Burridge and Halliday¹⁰ for an antiplane shear crack showing a stress-drop and frictional strength gradient with depth. However, such details may vary with a different choice of gradients.

In the second case, we allow a crack to nucleate in a lower stress-drop regime (Fig. 2*b*). This crack does not grow, which means that if an earthquake nucleates at shallow depths it is not as likely to propagate dynamically into the higher strength, deeper regions: only an earthquake that nucleates in the higher stress-drop part of the fault at depth has the potential to become a large or great earthquake (provided, of course, that other parts of the fault plane are close to failure). These results are independent of the numerical technique used in the calculations. A qualitatively similar result would be obtained from an analytical study of this problem using, say, a 'critical stress-intensity factor'¹¹ fracture criterion. The stress-intensity factor k for a crack is given by a relation of the form $k = C\Delta\sigma_{\text{dyn}}\sqrt{L}$, where $\Delta\sigma_{\text{dyn}}$ was defined above, L is the crack length and C is a constant depending on the shape of the crack and the crack velocity. This relation shows that the stress-intensity factor for a crack propagating in a lower stress-drop regime is lower than that of the same crack propagating in a higher stress-drop

regime. Thus, our numerical results indicate only that a crack propagating in a high stress-drop regime can propagate more easily into relatively lower strength regions than a crack in a low stress-drop regime can penetrate into relatively strong regions.

Direct evidence for this phenomenon is provided by the strong motion records of the 1971 San Fernando, California earthquake in which the region of slip extended about equally in depth and horizontal extent. The records indicate that the earthquake originated at depth with a very large stress-drop and propagated to the surface. During the latter stages of upward propagation, the inferred stress-drop associated with the fracturing was much smaller¹².

We have not included lateral variations in stress-drop and yield strength or other variations of stress-drop and strength with depth in our model. In general, such variations would exist, so that a crack nucleating at shallow depths might grow into a small earthquake but not into a major event. Because of the presence of heterogeneities, it is sometimes possible to observe a deeper earthquake having a smaller stress-drop than a shallower one in the same region¹³. There also appear to be rare large earthquakes that propagate downwards. One known example is the 1946 Aleutian earthquake¹. That earthquake had other peculiar properties also: despite being relatively small ($M_s = 7.4$), it generated the largest tsunami of this century in the Pacific. That rupture tends to propagate much more slowly downwards than upwards may be a clue to the high tsunami generation efficiency of an earthquake having a relatively small M_s .

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Abnormal floral development of a tobacco mutant with elevated polyamine levels

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We have previously isolated two mutant cell lines of *Nicotiana tabacum* defective in polyamine metabolism¹. One of these two cell lines, a temperature-sensitive mutant *ts4*, regenerated into light green plantlets with short internodes that could be maintained only in shoot cultures. The other cell line, *Rt1*, a revertant of the *ts4* line, also had short internodes, but was dark green, and produced flowers with a second row of petals in place of anthers². We describe here a second example of an *N. tabacum* mutant with altered polyamine metabolism and floral development: in this case a cell line selected as resistant to a spermidine synthesis inhibitor regenerates to give plants producing flowers with anthers in the place of ovules.

SDS-polyacrylamide gel electrophoresis^{3,4} of total protein extracts from wild-type, *ts4* and *Rt1* cells revealed a prominent band of molecular weight ~30,000 (30 K) present in the *ts4*

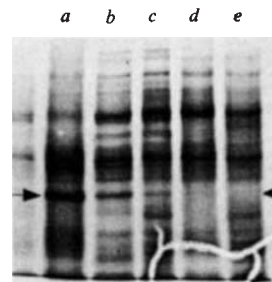


Fig. 1 SDS-polyacrylamide gel electrophoresis of polypeptides from wild-type, induced wild-type, and *ts4* cells. **a**, *ts4* cells in normal medium; **b**, wild-type cells grown in glutamine medium with 5 mM MGBG; **c**, wild-type cells in glutamine medium with 1 mM MGBG; **d**, wild-type cells in glutamine medium; **e**, wild-type cells in normal medium.

Methods: Liquid suspension culture cells were routinely grown in Murashige-Skoog basal medium⁶ with 3 mg indoleacetic acid and 0.3 mg kinetin added per litre, as previously described¹. Glutamine medium consisted of 30 mM glutamine substituting for the ammonium nitrate and potassium nitrate, since MGBG was insoluble in the presence of the high nitrate levels of the normal medium. Wild-type cells were subcultured by a fourfold dilution twice a week, and *ts4* was subcultured by a twofold dilution once a week. All were normally collected in early exponential phase. The wild-type cells to be induced were subcultured into the glutamine medium plus MGBG by washing the cells once, and following with a fourfold dilution. After 7 days the induced wild-type cells were subcultured either twofold or fourfold into fresh medium, and then collected 3 d later. Cells were collected by vacuum filtration, resuspended in 100 mM HEPES pH 7.5, 5 mM dithiothreitol, 0.1% SDS, boiled for 2 min, ground in a Sorvall Omni-mixer for 1 min, and the solution clarified by centrifugation at 12,000g for 15 min. The protein was precipitated with 9 volumes of acetone, and resuspended in 1/5 volume of SDS sample buffer. After a 2 min boil, the samples were loaded on a 12% polyacrylamide gel⁴.

cells, but not the wild-type or *Rt1* cells (Fig. 1). The polypeptide profiles are qualitatively the same when cells are grown at permissive (26 °C) or restrictive (33 °C) temperatures. However, when wild-type cells are grown in the presence of sublethal levels of the polyamine synthesis inhibitor methylglyoxal-bis (guanyldiazotone)^{5,6} (MGBG, which inhibits the enzyme S-adenosyl methionine decarboxylase, SDC) a 30 K polypeptide is induced (Fig. 1). Peptide mapping⁷ (Fig. 2) confirmed that this is the same polypeptide produced at high levels in the *ts4* cells.

The polypeptide phenocopy effect induced by MGBG suggested that mutants resistant to the inhibitor might have lesions related to those observed in *Rt1* and *ts4*. We isolated resistant lines by mutagenizing wild type diploid cells with UV light, and then selecting for growth on plates containing 10 mM MGBG. The UV light dose chosen gave 30% dead cells as assayed 24 h after irradiation by morbid staining with 0.1% bromophenol blue. Diploid cells of *N. tabacum* cv. Xanthi were grown in liquid suspension culture, irradiated, and then left to recover for one generation in the dark. MGBG was added and the cells grown for an additional generation in liquid culture, followed by plating on plates containing MGBG. Colonies that appeared were placed in liquid suspension culture and tested for growth and survival in MGBG.

One of the resistant lines obtained, *Mgr3*, regenerated relatively rapidly into a whole plant when placed in standard regeneration conditions¹. The plant was dark green, and had very short internodes. Leaves from the plant have been placed back into culture, and the resulting cell lines are fully resistant to the inhibitor. A plant regenerated from *Mgr3* has flowered, with several unusual characteristics. The flowers initially appeared normal, but then the stigma grew past the corolla by about 5 mm, and the stigma turned black. The anthers appeared normal initially, but never contained viable pollen, and died about the time the stigma grew past the corolla. The ovaries swelled

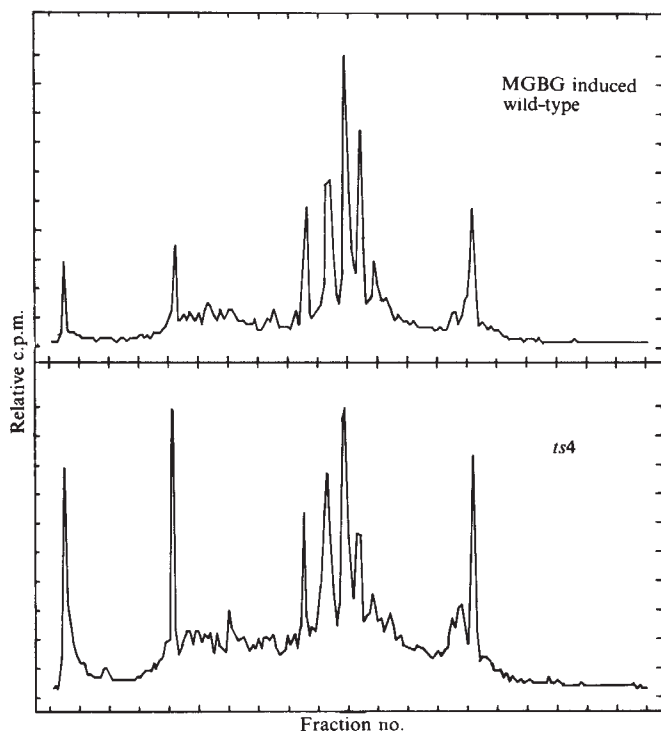


Fig. 2 Peptide maps produced by trypsin digests of the 30 K polypeptide.

Methods: Wild-type cells were grown for a week on glutamine medium with 5 mM MGBG as described in the legend to Fig. 1. They were then subcultured by a twofold dilution into fresh medium. A 10 ml sample was removed and 500 μ Ci of S^{35} -methionine (1,000 Ci mm^{-1} , NEN) was added. In 3 days the sample was collected as described previously. The *ts4* cells were also grown as previously indicated, a 10 ml sample removed and 500 μ Ci of S^{35} -methionine added. The *ts4* cells were grown for 5 days and collected. The denatured samples in SDS sample buffer were loaded on separate gels, with each sample covering the width of its gel. The gels were stained with Coomassie blue, dried and autoradiographed; a 12-h exposure gave a pattern equal in intensity to the corresponding stained bands. The tryptic digest peptide map was performed in the manner of Smart *et al.*⁷ on a Spectra-physics SP8000 HPLC system. 0.7-ml Fractions were collected yielding a total of 200 fractions. Aliquots of 175 μ l were taken from each fraction and mixed with 6 volumes of Aquasol for counting in a liquid scintillation counter. For purposes of comparative graphing, the highest peaks in each sample were normalized to an arbitrary 1,000 c.p.m. In fact, the highest peak in the MGBG induced wild-type sample was 2,389 c.p.m., and in the *ts4* sample was 1,206 c.p.m.

even though the plant had not been fertilized. Dissection of an ovary revealed that the hundreds of ovules normally found embedded in the placenta had been converted into hundreds of anthers (Fig. 3). Each ovary contained 5–10 ovules that did not switch to the male meiotic structure. In some flowers that were dissected at an early stage, we saw, between clusters of normal-looking ovules and transformed ovules, elongated spheres and tubular structures that were apparent intermediates between anthers and ovules. None of these structures could have resulted from fertilization, as *Mgr3* was male sterile, and there were no wild-type plants in flower in the same chamber.

We have dissected normal anthers and *Mgr3* ovule-anthers in order to compare their internal morphologies. Wild-type anthers have a characteristic internal morphology consisting of four columns of cells in a lobed structure, and similar structures were seen in cross-sections of the *Mgr3* ovule-anthers. The transformed ovules never form a well defined pollen sack, and never show pollen development, although the terminus is sometimes slightly enlarged with a dark green colour.

We have analysed the levels of polyamines in *Mgr3* cultures and leaves, and compared them with wild-type cultures and leaves on a nanomole per gram fresh weight basis (Table 1), using the method of Flores and Galston⁸. In *Mgr3* cultures, the levels of putrescine are relatively low, while in leaves from regenerated mutant plants the levels of spermidine and spermine are quite high. The ratio of putrescine to spermidine in wild-type cultures and leaves is about 1:1, whereas in *Mgr3* cultures and leaves it is 1:3 or 1:4. In *ts4* and *Rt1* cells the ratio of putrescine to spermidine is about 2:1. These values represent the free polyamine levels, and do not include the conjugated forms frequently found in plants⁹.

Recently a second MGBG resistant mutant, *Mgr12*, has been regenerated into a whole plant, and has flowered. The flowers are nearly normal in gross morphology, but the plant is male sterile with an absence of pollen formation. The plant is female fertile, and has been fertilized with wild-type pollen.

That several independent mutants have been isolated that are affected in polyamine synthesis and also have altered expression of floral meristems suggests that they are related in some way. However, it may not be that the relationship is a direct and specific one, it may simply be that flower development is very sensitive to metabolic perturbations. Alternatively it is possible that polyamines have a specific role in development: perhaps gradients of polyamines are involved in determining the developmental fate of floral meristems. No clear understanding has yet emerged of the role of polyamines in plant physiology, although there are several reports of polyamine synthesis changing in response to hormones^{10,11} and phytochrome regulation^{12,13}. Heimer and Mizrahi¹⁴ have shown that the enzyme ornithine decarboxylase (which catalyses putrescine synthesis from ornithine) is expressed at high levels in tobacco cell cultures and tomato ovaries, and that the levels vary during the cell

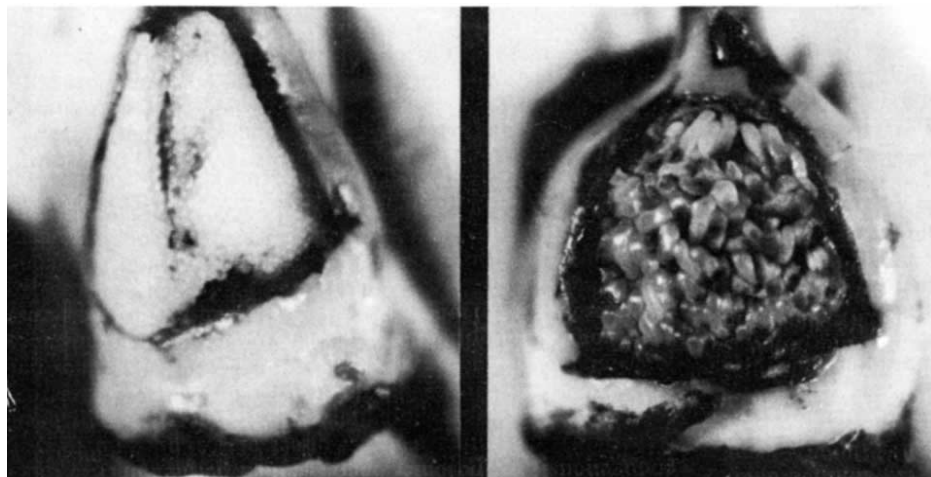


Fig. 3 Dissections of wild-type (left) and *Mgr3* (right) ovaries, showing the white disk-shaped ovules of wild type, and the green ovule into another transformation of *Mgr3*.

Table 1 Polyamine concentrations of *Mgr3* and wild-type cells

Cell type	Concentration (nmol g ⁻¹)		
	Putrescine	Spermidine	Spermine
Wild-type culture	480 (38)	430 (53)	49 (3)
Wild-type leaf	410 (68)	430 (24)	23 (5)
<i>Mgr3</i> culture	180 (27)	520 (27)	50 (5)
<i>Mgr3</i> leaf	570 (36)	2,030 (89)	160 (7)

Free polyamine concentrations were measured in the manner of Flores and Galston⁸, by chromatographing benzoylated extracts on a Waters 720 liquid chromatography system on a 4.6 mm × 25 cm Spherisorb 10 ODS (C18) reverse phase column. The column was run at room temperature at a flow rate of 1 ml min⁻¹ isocratically in 60% methanol, and absorbance monitored at 254 nm. Cell cultures were collected by vacuum filtration and weighed; leaves were simply weighed. The samples were then homogenized with a Polytron (Brinkmann) with all other manipulations as described by Flores and Galston⁸. The values in parentheses are the standard errors as determined by four replications.

cycle. Thus, there are hints that polyamines may have an interesting role in development, an idea supported by our observations with tobacco mutants.

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Long-term culture of murine erythropoietic progenitor cells in the absence of an adherent layer

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Replication of multipotential stem cells in long-term murine bone marrow cell culture is known to depend on the development of an adherent stromal cell layer. In these conditions, restricted haematopoietic progenitor cells have also been generated for up to several months^{1–3}. However, maturation is observed only in the granulocyte/macrophage and megakaryocyte lineages; erythropoiesis appears to be blocked at the earliest burst-forming unit (BFU-E) stage. Addition of exogenous erythropoietin (Epo) or anaemic mouse serum results in full erythropoietic maturation, but it is transient^{4,5}. We describe here a culture system in which production of erythropoietic progenitor cells can be maintained for over 6 months in the absence of an adherent stromal layer and in the absence of added Epo, but in the presence of pokeweed mitogen-stimulated spleen cell conditioned medium (PWSCM). The data indicate that restricted erythroid progenitor cells exist which are capable of extensive self-renewal.

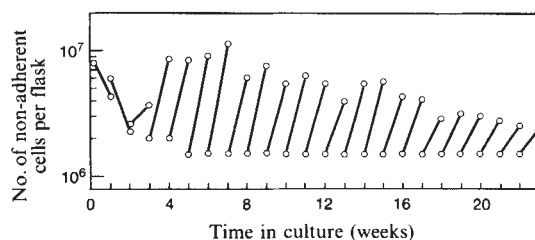


Fig. 1 Growth of the nonadherent cell population. Cultures were initiated with pooled femoral marrow cells (from 8–10-week-old female C57BL/6J Ut mice) suspended in alpha medium supplemented with 20% heat-inactivated FCS (Flow, Lots 29101248 and 29101406), 1% deionized bovine serum albumin (Fraction V, Sigma), 10% PWSCM, penicillin (100 U ml⁻¹) and streptomycin (50 µg ml⁻¹). Aliquots of 8 ml of cell suspension (1 × 10⁶ cells ml⁻¹) were cultured in 25-cm² plastic flasks (either Corning or Falcon) and incubated in a humidified atmosphere of 5% CO₂ in air at 33 °C. After 1 week of incubation the nonadherent cells were collected from 8–10 flasks and pooled. Medium was totally removed and cells were transferred at the concentrations indicated into fresh flasks containing fresh medium. This transfer procedure was repeated at weekly intervals. PWSCM was prepared following the method described by McLeod *et al.*⁶. Spleen cells from C57BL/6J Ut mice were suspended at a concentration of 3 × 10⁶ cells ml⁻¹ in NCTC 109 medium containing 10% FCS and PWM 1:300 (Gibco). Aliquots of 20 ml were incubated at 37 °C in a humidified atmosphere of 5% CO₂ in air. After 4 days, the medium was collected, centrifuged (1,700g, 20 min) to remove cells and debris and the supernatant was stored at –20 °C. Batches were prescreened for their ability to enhance erythropoietic burst production in the plasma culture system. One of seven such experiments is illustrated.

Cultures of C57BL/6 mouse bone marrow cells were initiated and maintained as described in Fig. 1 legend. During the first few passages, numerous cells became attached to the culture flasks; however within four to five passages, adherent cells were totally eliminated, and the cells in suspension continued to proliferate for over 6 months.

To investigate the erythropoietic progenitor cells in the cultures, cells were seeded in semi-solid medium containing Epo⁶. Very few day-2 colony-forming units (CFU-E) were detected. However, by day 3, the colony number ranged from 24 to 105 per 10⁵ cells (Fig. 2).

During the first 3–4 weeks of culture, we observed a decrease in frequency of BFU-E; thereafter, their concentration increased and reached a level of 90–120 BFU-E per 10⁵ cells. Table 1 shows the data for one representative cell line (seven cell lines produced in seven attempts). The time of increase in BFU-E frequency seems to correlate with the time of disappearance of the adherent cell population. The highest frequencies of day-4 and day-7 BFU-E were detected between weeks 5 and 10. When the suspension cultures were assayed for day-10 BFU-E, only a small number of erythroid colonies produced could be scored as bursts, since most of them were composed of fewer than 50 haemoglobinized cells.

We next investigated the effect of adding PWSCM^{7–10} to the Epo-containing culture medium. It had no effect on the number of day-4 bursts (Table 1). However the day-7 and day-10 bursts were increased 2- to 20-fold compared with cultures stimulated by Epo alone. The total cellularity within each burst was also greatly increased: an estimated 5 × 10²–5 × 10³ haemoglobinized erythroblasts made up the majority of bursts.

When suspension cultures were kept for 7 days in the absence of PWSCM, most of the cells died and no erythroid progenitors could be detected. In the presence of PWSCM, large numbers of erythropoietic progenitors from day-10 BFU-E to day-3 CFU-E continued to be generated for many months in the absence of an adherent layer. Given that the cells in suspension were reseeded each week at low density, the increase in BFU-E above input numbers must represent true amplification. This process evidently requires a factor(s) present in PWSCM; terminal maturation (haemoglobinization) of the CFU-E progeny

Table 1 Erythropoietic burst formation by suspension cultures

Weeks cultured	Day 4 BFU-E		Day 7 BFU-E		Day 10 BFU-E	
	Epo	Epo+PWSCM	Epo	Epo+PWSCM	Epo	Epo+PWSCM
0	34.5±7.4	32.0±6.1	39.0±8.2	42.6±9.3	27.6±5.1	ND
3	15.3±1.5	15.6±1.4	5.0±2.3	ND	7.7±1.0	31.4±3.0
5	ND	ND	91.0±10.9	Confluent	ND	ND
6	32.8±7.6	30.0±4.3	111.0±20.2	147.6±28.6	18.2±2.5	146.0±16.8
7	31.5±4.9	33.4±8.3	40.0±8.0	90.0±25.4	22.0±7.8	Confluent
8	106.0±21.1	ND	7.2±2.3	46.2±13.6	4.4±1.6	Confluent
9	90.2±11.3	ND	17.6±6.4	34.8±10.0	3.2±2.5	65.8±12.7
10	46.0±6.1	39.5±8.6	45.0±5.8	66.4±5.4	10.6±3.0	76.5±9.6
11	6.5±4.1	7.0±2.8	1.2±0.5	24.7±6.1	ND	ND
12	10.4±3.0	ND	3.5±1.5	39.0±9.7	4.0±0.4	45.5±13.2
14	18.2±2.5	ND	4.5±1.7	18.4±5.8	7.6±4.1	51.6±11.7
16	21.2±6.3	ND	1.2±1.2	14.0±4.8	0	19.2±7.0
18	43.2±2.7	ND	15.0±4.5	25.0±3.5	ND	ND

Clonal assays for erythropoietic progenitor cells were performed in the plasma culture system. On the weeks indicated, cells from one cell line in suspension culture were plated at a concentration of 1×10^5 cells per 0.5 ml with Epo alone (1.5 U per 0.5 ml) or at 5×10^4 cells per 0.5 ml with Epo (1.5 U per 0.5 ml) and PWSCM (0.5% final). Plasma cultures were collected after incubation at 37 °C for 4, 7 and 10 days. Day-4 bursts were counted as colonies consisting of three or more clusters of a haemoglobinized erythroblasts. Day-7 and -10 bursts were scored as colonies comprising more than 50 erythroblasts. Data (mean ± s.d. for quadruplicate cultures) are expressed as the number of bursts per 10^5 cells. ND, not determined.

Table 2 Titration of conditioned medium on normal bone marrow cells

% CM	BFU-mix/E	PWSCM		WEHI-3 conditioned medium		
		MK-CFC	GM-CFC	BFU-mix/E	MK-CFC	GM-CFC
0	18.1±3.6	11.8±4.0	15.6±1.6	15.3±4.2	7.3±1.8	18.5±3.5
0.625	25.0±2.4	23.5±3.2	30.3±8.8	ND	ND	ND
1.25	26.8±1.8	23.3±3.8	47.6±5.6	30.2±7.4	16.0±1.4	60.2±5.6
2.5	29.6±4.8	27.1±1.4	45.3±7.9	39.6±5.8	15.6±2.8	92.0±12.2
5	32.8±6.2	25.3±3.8	51.6±7.2	40.1±4.6	18.1±3.2	109.1±12.0
10	32.3±3.8	22.3±1.6	57.8±6.0	34.6±4.0	19.0±3.2	106.8±12.2
20	25.7±3.2	20.5±2.4	47.1±7.6	30.1±7.4	17.2±2.8	137.5±7.0

5×10^4 C57BL/6 bone marrow cells were plated in the plasma culture system in the presence of 1.5 U Epo per 0.5 ml and serial dilutions of conditioned medium. PWSCM was prepared as described in Fig. 1 legend. WEHI-3 cells were passaged continuously in IMDM (Iscove-modified Dulbecco medium, Gibco) containing 2% fetal calf serum (FCS). Medium was collected when growth had ceased and cells were beginning to die. Plasma cultures were collected after 7 days of incubation at 37 °C. They were stained with benzidine and counterstained with haematoxylin. Numbers of pure erythrocytic and mixed erythrocytic colonies combined (BFU-mix/E), pure megakaryocytic and mixed megakaryocytic/erythrocytic colonies combined (MK-CFC) and pure granulocytic and mixed granulocyte/macrophage colonies combined (GM-CFC) were determined from six replicate cultures at each point (mean ± s.d.). ND, not determined.

requires Epo. These findings provide strong support for the two-regulator model of erythropoiesis⁸⁻¹².

After week 6 of culture, when cells were plated in semi-solid medium in the presence of Epo (3 U ml⁻¹) and PWSCM (0.5%), each burst appeared to be composed of only erythroblastic cells at different stages of maturation. No megakaryocyte, granulocyte-macrophage or mixed erythroid colonies—either bilineal (megakaryocyte-erythrocyte) or trilineal (megakaryocyte-granulocyte-erythrocyte)—were observed. To ensure that conditions were optimal for detecting these progenitors, cells from suspension cultures were plated with increased concentrations (5% and 10%) of either PWSCM or WEHI-3-cell conditioned medium. But even in these conditions, we could not detect such progenitors in the long-term cultures despite the fact that, when titrated on normal bone marrow cells, the conditioned media stimulated two- to sixfold the production of granulocyte-macrophage, megakaryocyte and mixed erythroid colonies (Table 2). The loss of stromal cells presumably deprived granulocytic and megakaryocytic progenitors of factor(s) necessary for their survival and proliferation in liquid culture. These factor(s) could not be replaced by PWSCM, although this medium contains factors favouring the production of both granulocyte and megakaryocyte colonies in semi-solid culture. In contrast, the loss of stromal cells did not impair—and may even have favoured—the survival and proliferation of erythroid progenitor cells in suspension culture. The factor(s) essential for the growth of these progenitors must thus be provided by PWSCM.

From week 6 to week 14, cultured cells were injected intravenously into pre-irradiated syngeneic mice (9 Gy, ¹³⁷Cs

γ rays). Hosts' spleens were examined 9–10 days later¹³. No spleen colonies could be detected even when 2×10^6 cells were injected into each recipient mouse. Serial sections of the spleen confirmed that no intrasplenic or microscopic colonies were present. Multipotential haematopoietic stem cells capable of forming spleen colonies in irradiated hosts (CFU-S) or mixed erythroid colonies *in vitro* thus appeared to be lost after a few weeks in our suspension cultures.

Three lines of evidence argue against the possibility that these erythroid progenitor cells have been transformed. (1) They have an absolute requirement for a factor(s) in PWSCM for their growth and Epo for their terminal maturation. (2) No significant reverse transcriptase activity was detected in supernatants of 2/2 of the cell lines tested. (3) No tumours or leukaemias developed over an 8-month period when the cells were injected intravenously, intraperitoneally or subcutaneously into either unirradiated or sublethally irradiated (5 Gy) syngeneic hosts.

The generation of erythropoietic progenitors could be sustained in the absence of detectable multipotential stem cells. This suggests that our culture conditions might have selected for a restricted erythroid progenitor cell capable of extensive self-renewal. Similar factor-dependent haematopoietic progenitor cell lines, either multipotential¹⁴ or restricted to the granulocytic lineage¹⁵, have been described.

Erythropoietic progenitor cells represented only a minority (0.1–0.2%) of the population in long-term culture. In cytopsin preparations, the cells were found negative for staining with myeloperoxidase, acetylcholinesterase, nonspecific esterase, benzidine, luxol fast blue and oil red O. No cells reacted with monoclonal anti-Thy 1.2 antibodies or anti-spectrin antibodies.

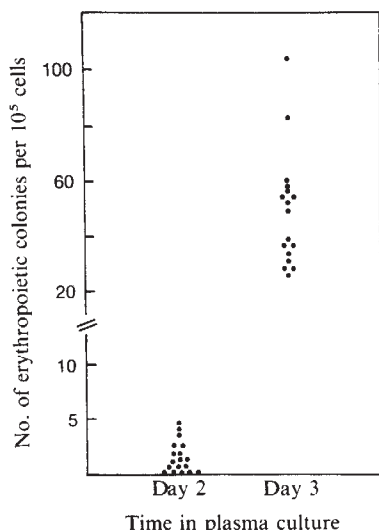


Fig. 2 Formation of CFU-E colonies by cells from suspension cultures. Cells were plated in the plasma culture system at a concentration of 1×10^5 cells per 0.1 ml in the presence of 0.3 U ml^{-1} Epo (sheep plasma erythropoietin, Step III, Connaught). Plasma cultures were incubated at 37°C in a fully humidified atmosphere of 5% CO_2 in air. Clots were collected on days 2 and 3. Clusters containing at least eight benzidine-positive erythroblasts were scored as CFU-E colonies. Each point represents the mean number of CFU-E colonies for six clots (results of 17 experiments carried out between weeks 7 and 18).

However, we observed a progressive increase in the proportion of cells showing metachromatic granules with toluidine blue¹⁶, from less than 1% during the first week to over 90% in the fifth week of culture. Preliminary studies indicate that these cells contain low amounts of histamine and therefore appear to be similar to the mast cells described in continuous cultures of haematopoietic tissues^{17–21}. Their precise nature is being investigated. The prolonged association of erythroid progenitor cells with mast-like cells in these cultures raises the possibility of a bipotential progenitor restricted to erythroid and mast cell lineages. Clonal analysis of these cell populations should provide further information.

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Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin

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Arteries relax to the vasodilators acetylcholine, substance P, ATP and bradykinin only if the endothelium is present^{1–4}. One hypothesis is that these substances stimulate the endothelial cells to release a vasodilator substance which in turn relaxes the underlying smooth muscle. We considered that other hormones which have direct actions on smooth muscle cells may also release the dilator substance. If the hormone contracts smooth muscle cells and also activates the release of the dilator from endothelial cells, the algebraic sum of these stimuli would determine the physiological response. Our preliminary experiments in pig and dog isolated coronary arteries showed that noradrenaline (NA) and serotonin (5-hydroxytryptamine, 5-HT) were significantly more powerful vasoconstrictors in the absence of endothelium. We report here the unexpected finding that these constrictor amines release a vasodilator substance from endothelial cells that can act as a physiological antagonist of the well known smooth muscle contractile responses. We suggest that the potential involvement of the vasodilator signal should be considered in the responses to vasoconstrictors in both normal and diseased blood vessels.

The circumflex coronary artery was carefully isolated from freshly (5 min) dissected hearts from male and female pigs (Large White; 20–25 kg), greyhound dogs (20–30 kg) or mongrel dogs (15–20 kg). The arteries were cut into 3–4-mm long ring segments and were suspended in 30-ml organ baths containing Krebs' solution maintained at 37°C and bubbled continuously with 95% O_2 , 5% CO_2 (ref. 5). Changes in isometric force were recorded as described previously⁵. In all experiments in which noradrenaline or adrenaline were used, EDTA ($40 \mu\text{M}$) was included to inhibit oxidation.

The endothelium was removed from some rings by gently rubbing the intimal surface with a filter paper taper moistened with Krebs' solution. This procedure removed >90% of the endothelial cells and caused little if any damage to the underlying smooth muscle as observed from both *en face*, silver-stained whole-mount sections for light microscopy⁶ and thin sections for electron microscopy. Furthermore, as a check on the functional removal of the endothelium, the responsiveness of each preparation was tested to a known endothelium-dependent dilator, substance P. In all endothelium-intact rings of artery, substance P (3 nM) inhibited the contractions induced by 5-HT, NA or U46619 (a thromboxane A_2 mimetic) by 90–100% and those induced by K^+ by 30–50%. However, in all precontracted rings devoid of endothelium, substance P (3 nM) had no effect. This finding was not due to the inability of the tissue to relax, because an endothelium-independent dilator, papaverine ($3 \mu\text{M}$), caused >90% relaxation in each case.

Removal of the endothelium from both dog and pig coronary artery rings shifted the contraction curves to 5-HT and NA to the left and increased their respective maxima compared with those of rings in which the endothelium remained intact (Fig. 1). Preliminary data from rat thoracic aorta also suggested that endothelium removal enhanced the sensitivity to NA⁷. In contrast, the contraction curves for U46619 were unaffected and

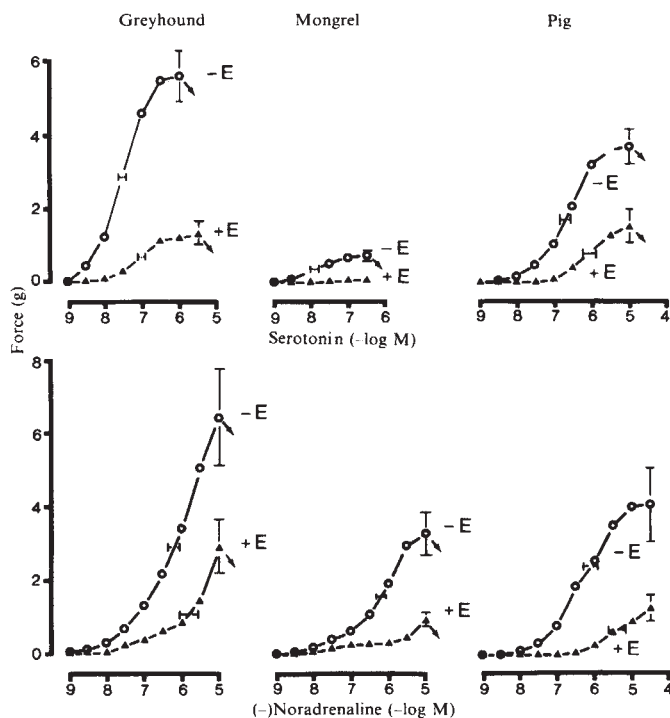


Fig. 1 Effect of endothelial cell removal on the contractile responses to (-)-noradrenaline and serotonin (5-HT), in greyhound, mongrel dog and pig isolated circumflex coronary artery. Rings of artery were prepared with their endothelium intact (+E) or removed (-E) as described in the text. From each artery up to 12 ring segments were obtained, 6 with and 6 without endothelium. These rings were allocated to one of four constrictor substances, NA (in the presence of propranolol, $1 \mu\text{M}$), 5-HT or K^+ and U46619 (see Fig. 2). Cumulative concentration-contraction curves were constructed and EC_{50} values (horizontal bars: $-\log$ molar concentration; mean $\pm$ s.e.m.) were obtained by computerized sigmoid curve fitting analysis¹⁷. Each curve comprised data from at least five rings.

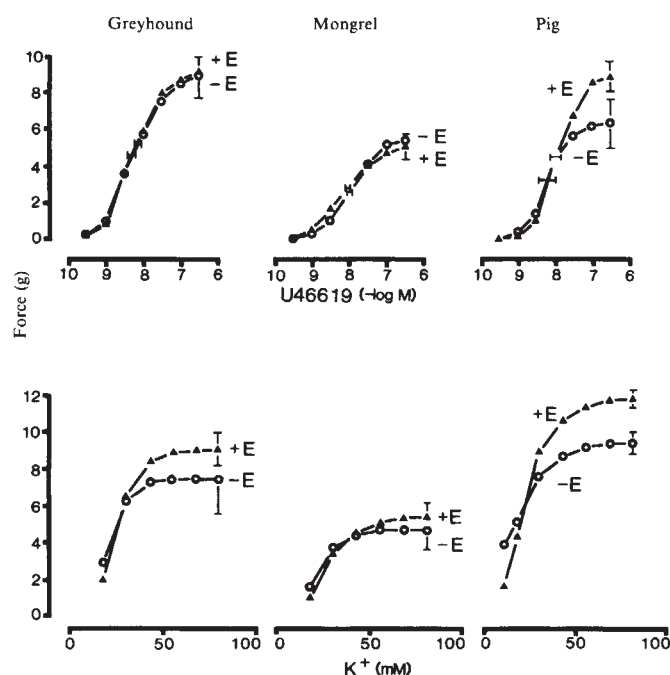


Fig. 2 Effect of endothelial cell removal on the contractile responses to U46619 and KCl (K^+) in greyhound, mongrel dog and pig isolated circumflex coronary artery. The preparation of the rings and construction of the contraction curves were as described in Fig. 1 legend. Note that for the K^+ curves the concentration of K^+ (ordinate) is plotted on a linear scale (mM).

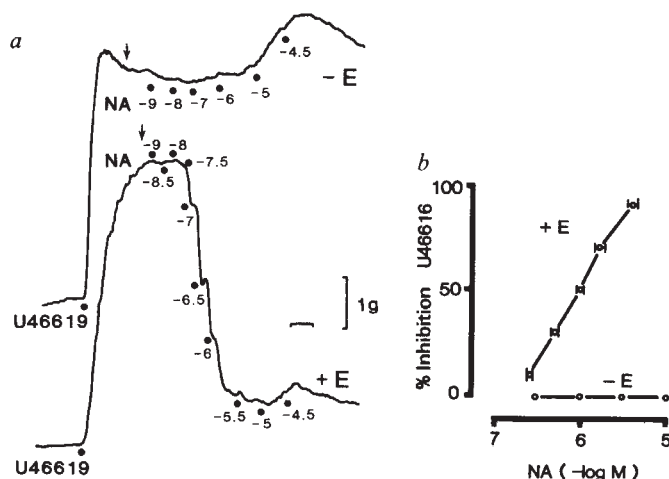


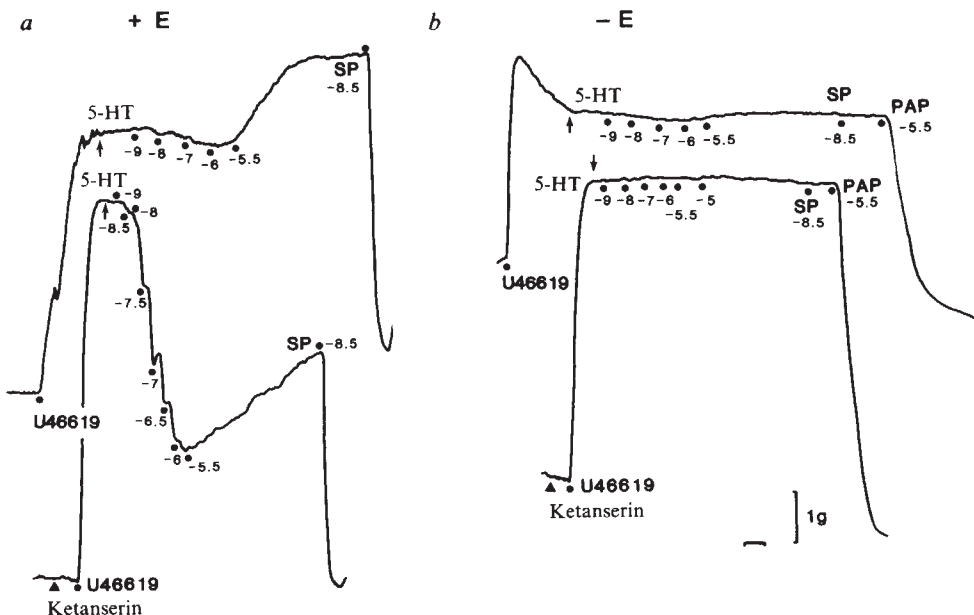
Fig. 3 α -Adrenoreceptor-mediated relaxation of pig circumflex coronary artery. *a*, Chart recordings from two rings of coronary artery, one with and one without endothelium, each incubated with propranolol ($3 \mu\text{M}$) and EDTA ($40 \mu\text{M}$) for 10 min before the addition of U46619 (10 nM). When the contraction to U46619 had reached a steady level, (-)noradrenaline (Na: $\log \text{M}$) was added cumulatively to each tissue. In the ring with an intact endothelium (+E), NA caused a concentration-dependent relaxation. In the ring without endothelial cells (-E) no inhibition by NA occurred, only contraction at higher NA concentrations. The time scales before and after the addition of NA are different. The horizontal calibration bar represents 12 min before and 2 min after the arrow. *b*, Pooled data. Mean values $\pm$ s.e.m. of $\log \text{IC}_{30}$, IC_{50} , IC_{70} and IC_{90} were determined from 12 rings in at least 5 animals. Propranolol ($3 \mu\text{M}$) and prazosin ($1 \mu\text{M}$) were present throughout. Rings contracted with U46619 maintained a steady plateau of force for up to 1 h when no other drugs were added (see ref. 18).

those for K^+ were decreased by the removal of the endothelium (Fig. 2). The reason for the latter response is unknown.

These findings suggest that NA and 5-HT, but not U46619 or K^+ , release a vasodilator substance from endothelial cells. To examine this for NA, pig coronary artery rings with and without endothelium were precontracted with U46619 (10 nM) in the presence of propranolol ($3 \mu\text{M}$), to block any inhibitory β -adrenoceptors, and in some cases with prazosin ($1 \mu\text{M}$), to block excitatory α_1 -adrenoceptors (both located on smooth muscle). In these conditions NA (0.01 – $10 \mu\text{M}$) caused a concentration-dependent relaxation only in those rings in which the endothelium remained intact (Fig. 3). Adrenaline, also in the presence of propranolol (1 – $3 \mu\text{M}$), caused concentration-dependent relaxation of pig coronary arteries with a similar potency to that of NA. Like NA, the relaxation was also dependent on an intact endothelium. Methoxamine (0.1 – $30 \mu\text{M}$), a selective α_1 -adrenoceptor agonist, did not elicit the inhibitory response, but only caused contraction which was blocked by prazosin (1 – $3 \mu\text{M}$). NA also caused concentration-dependent relaxations of U46619-contracted coronary arteries from the mongrel and greyhound which, like those in the pig, were dependent on the endothelium. However, in the greyhound, the inhibitory response was only observed in the presence of prazosin (1 – $3 \mu\text{M}$) whereas it was observed in both the mongrel dog and pig without added prazosin (Fig. 3a). In two greyhounds, clonidine (3 – $30 \mu\text{M}$), a selective α_2 -adrenoceptor partial agonist, caused a small relaxation which again was dependent on an intact endothelium.

We attempted to classify the α -adrenoceptor on the endothelial cell. The endothelium-dependent relaxation curve to NA in the pig coronary artery was shifted to the right in both a concentration-dependent and parallel manner by the α -adrenoceptor antagonist phentolamine. Dissociation constants (pK_B)⁸ for phentolamine at 0.1 and $1.0 \mu\text{M}$ were 7.39 ± 0.09 and 7.18 ± 0.06 , respectively (mean $\pm$ s.e.m. from seven observations in five pigs). In a similar assay the more selective α_2 -adrenoceptor antagonist, RX781094 (Reckitt & Coleman)⁹

Fig. 4 Chart recordings depicting relaxations of rings of pig isolated circumflex coronary artery to 5-HT and its dependence on endothelium. *a*, Rings with endothelium (+E); *b*, rings without endothelium (-E). All rings were precontracted with U46619 (10 nM) and 5-HT was added cumulatively in the concentrations (-log M) indicated. Ketanserin (3 μ M) was added 10 min before U46619 at Δ . Ketanserin unmasked a concentration-dependent relaxation to 5-HT only in the +E ring. Also, substance P (SP; 3 nM) caused maximal or near maximal relaxation in both +E rings, but no response in the -E rings. However, the -E rings could relax maximally to papaverine (PAP; 3 μ M). The time scales before and after the addition of 5-HT are different. The horizontal calibration bar represents 12 min before and 2 min after the arrow.



gave pK_B values for 0.1 and 1.0 μ M of 7.55 ± 0.04 and 7.30 ± 0.03 , respectively (mean $\pm$ s.e.m. from five observations in four pigs). Another selective α_2 -adrenoceptor antagonist, yohimbine (1–3 μ M), also antagonized NA-induced relaxation. These data suggest that the α -adrenoceptor is consistent with the α_2 subclassification.

It is unlikely that the vasodilator responses to NA and adrenaline are due to activation of inhibitory β -adrenoceptors located on smooth muscle cells. The β -adrenoceptor antagonist propranolol was present throughout all the experiments and at concentrations of 1.0 and 3.0 μ M it caused approximately 100- and 500-fold parallel rightward shift of the relaxation curve to isoprenaline (a β -adrenoceptor agonist) in pig and greyhound coronary arteries. Also, the relaxation to both NA and adrenaline was completely abolished by removal of the endothelium.

5-HT also caused an endothelium-dependent relaxation of coronary arteries in a similar manner to that just described for NA. For example, relaxation of the mongrel dog coronary artery to 5-HT was readily observed, but only in the presence of an intact endothelium. However, in the pig coronary artery, a concentration-dependent relaxation response to 5-HT was unmasked only by pretreatment with the S_2 (5-HT $_2$)-receptor antagonist¹⁰ ketanserin (1–10 μ M) (Fig. 4a) and this response was also abolished following removal of the endothelium (Fig. 4b). An explanation for this is that 5-HT can activate smooth muscle S_2 -receptors (contraction) in opposition to the endothelium-dependent relaxation.

The subclass of 5-HT receptors on endothelial cells which mediate smooth muscle relaxation remains unknown. However, they are unlikely to be S_2 -receptors because ketanserin accentuated rather than blocked the relaxation response to 5-HT in the coronary artery. Also, it is generally accepted that S_2 -receptors are located on vascular smooth muscle cells and mediate contraction.

NA and 5-HT also caused endothelium-dependent relaxations in pig renal and mesenteric artery rings which were either precontracted with U46619 or displayed their own inherent force. In both arteries, 5-HT (0.001–1.0 μ M) caused concentration-dependent relaxations which were enhanced by ketanserin (3 μ M). NA caused concentration-dependent contractions in both arteries. However, incubation with prazosin (3 μ M) abolished the contractions and unmasked concentration-dependent relaxations, which again were absent after removal of the endothelium.

Our findings suggest that stimulation of α_2 -adrenoceptors and 5-HT receptors (not S_2) located on endothelial cells lining several large arteries, including coronary, mesenteric and renal,

cause these vessels to relax. The mechanism for this endothelium-dependent relaxation is unknown, but is probably similar to that described for other vasoactive humoral agents such as acetylcholine, substance P, ATP and bradykinin^{1–3}. The physiological significance of such a dilator is also unknown. The endothelium-dependent relaxation to vasoconstrictors observed in these large arteries may also occur in resistance arterioles. One possible role, therefore, is that the inhibitory signal acts as a physiological antagonist to circulating or locally released neural vasoconstrictors and thus may be important in the control of blood flow. There may be both species and tissue variation in the presence of α_2 -adrenoceptors and 5-HT receptors on endothelial cells similar to differences in the distribution of endothelial receptors for dilators such as substance P, acetylcholine and bradykinin^{1,2}. This may help to explain the apparent lack of effect of endothelium removal of the contraction curve to NA in the dog isolated femoral artery³.

Attenuation or loss of the endothelial vasodilator response may also contribute to certain pathological conditions. For example, in variant angina there appears to be intense focal vasoconstriction, perhaps in areas of endothelial cell loss^{11,12}. It is interesting that a diagnostic test for patients with variant angina is their sensitivity to ergometrine. In these patients ergometrine given intravenously causes severe focal coronary vasospasm whereas in normal patients only a diffuse, limited vasoconstriction is observed^{13–15}. Ergometrine is a potent 5-HT-receptor agonist in canine coronary artery⁵. Thus, an altered reactivity to 5-HT *in vivo* may be an important underlying factor in the mechanism of coronary vasospasm. The present studies clearly show that loss of either endothelial cells or even their ability to produce and release the vasodilator signal, could account for such an altered reactivity to 5-HT. Interestingly, atherosclerotic strips of rabbit thoracic aorta have been reported to be more sensitive to ergometrine than normal strips¹⁶, although no details were given concerning the integrity of the endothelium in these experiments. A similar endothelium-dependent altered sensitivity to catecholamines may also contribute to vasospasm.

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Specific suppression of responses to *Leishmania tropica* by a cloned T-cell line

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Most clinical cases of cutaneous leishmaniasis caused by *Leishmania tropica major* consist of self-healing lesions that are associated with the development of strong specific cell-mediated immunity (CMI) detected by delayed-type hypersensitivity (DTH)^{1,2}. At the other extreme are susceptible individuals who develop persistent or diffuse forms of the disease. This spectrum of cutaneous leishmaniasis can be reproduced in in-bred mouse strains according to their genetic constitution³. BALB/c mice are exceptionally susceptible: the disease can be induced with minimum infecting doses and is inexorably progressive, terminating in cutaneous and fatal visceral metastasis^{3–7}. In this case, potentially curative CMI is abrogated by the generation of a specific Lyt-1⁺2⁺, I-J⁺ population of suppressor T (T_s) cells^{8,9}, whose induction is probably secondary to a primary macrophage defect^{10,11} controlled by a single major non-H-2-linked autosomal gene^{5,6}. The generation of the T_s cells can be prevented and infection cured by prior sublethal (550 rad) irradiation⁸. The potency of the T_s cells is such that as few as 10⁶ cells transferred adoptively can completely reverse this prophylactic effect⁹. In view of their potency and potential relevance to clinical leishmaniasis, I have now studied T_s cells at the clonal level to delineate further their functional characteristics and interaction with the effector mechanism. I report here a cloned T-cell line expressing specific suppression against *in vitro* lymphocyte proliferation and *in vivo* induction of DTH to *L. tropica* antigens.

L. tropica-specific T_s cells were derived from spleen-cell populations of BALB/c mice infected 69 days previously with 2 × 10⁷ promastigotes. The donor mice showed no DTH response but strong T_s reactivity as described previously⁸. The cells were cultured with irradiated syngeneic spleen cells and formalin-fixed *L. tropica* promastigotes (FFP) in medium containing 10% fetal calf serum and 20% T-cell growth factor (TCGF) as described in detail in Table 1 legend. After more than 2 months of continuous culture, T_s cells were cloned by limiting dilution in Terasaki plates.

One of the clones (LTC5) which showed vigorous growth was expanded, as described in Table 1 legend, and tested for suppressive activity against the induction of DTH to *L. tropica*. Normal BALB/c mice were injected intravenously (i.v.) with 10⁷ LTC5 cells or 0.5 ml supernatant from LTC5 culture. Control mice were given either 10⁸ normal T cells or 0.5 ml supernatant from culture without LTC5. All mice were immunized subcutaneously in the flanks with 4 × 10⁷ FFP immediately after cell or supernatant transfer. DTH reactivity was elicited with 10⁷ FFP in the footpad 7 days after immunization. Mice given LTC5 cells or LTC5 supernatant produced significantly lower levels of DTH to *L. tropica* compared with controls (Table 1). The suppression was antigen specific since no effect was evident against the induction of DTH to a non-cross-reacting antigen, sheep red blood cells (SRBC). The suppression is also unlikely

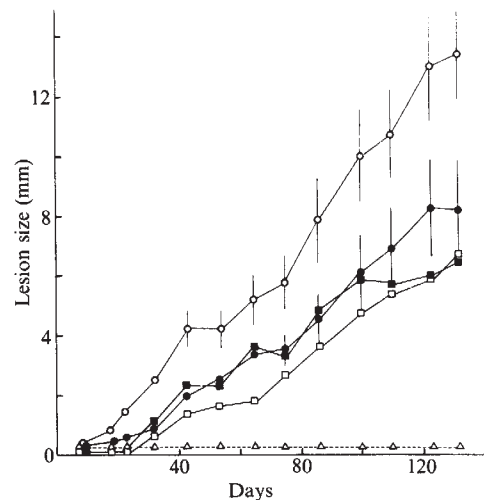


Fig. 1 Groups of BALB/c mice were injected on day 0 as follows: 5 × 10⁶ LTC5 cells mixed with 2 × 10⁴ promastigotes s.c. (○); 50 µl LTC5 supernatant mixed with 2 × 10⁴ promastigotes s.c. (■); 2 × 10⁷ LTC5 cells i.v. + 2 × 10⁴ promastigotes s.c. (□); 2 × 10⁴ promastigotes alone s.c. (●); and 5 × 10⁶ LTC5 cells alone s.c. (△). All s.c. injections (0.1 ml) were administered into the shaved rump. The lesions that developed were measured at intervals up to 135 days with a direct-reading caliper gauge (GMH-390 T Gallenkamp, London) in two perpendicular diameters. The average diameter (mm) was recorded and corrected for the thickness of the skin at the same site of an uninfected mouse, *n* = 4–9; vertical bars denote 1 s.e. of the mean.

to be due to carrying over an excess of antigens (FFP) because the control supernatant from a culture containing the same amount of FFP and irradiated spleen cells was without effect. Similar results were obtained in two further experiments using LTC5 cells and their supernatants 3–4 months after the cloning.

The suppressive activity of LTC5 and its product was also tested against the *in vitro* proliferation of two effector T-cell lines, TD and TDA. TD line was derived from lymph-node cells of a BALB/c mouse which had recovered from an *L. tropica* infection as a result of prior sublethal irradiation. It had been maintained in continuous tissue culture with FFP and TCGF for over 2 months. TDA line was derived from the spleen of a BALB/c mouse immunized subcutaneously (s.c.) with 10⁸ 2,000-rad-irradiated CBA spleen cells. TDA had also been maintained in culture with irradiated CBA spleen cells and TCGF for over 2 months. Both TD and TDA lines are capable of mediating passive local transfer of DTH into normal BALB/c mice. They are antigen specific in proliferation and grow independently of exogenous TCGF. LTC5 cells, however, are totally TCGF dependent and are not able to proliferate in the presence of antigen and feeder cells alone (Table 2). Furthermore, when LTC5 cells or their culture supernatant were added to TD culture, they significantly inhibited the proliferation of TD cells. The suppression is antigen specific since neither LTC5 cells nor their products affect the *in vitro* proliferation of TDA cells (Table 2).

The functional activity of LTC5 cells and their product was also tested *in vivo* against *L. tropica* infection. Normal BALB/c mice were infected s.c. with 2 × 10⁴ promastigotes and injected with LTC5 cells or LTC5 culture supernatant (Fig. 1). The ensuing lesion development was followed for up to 135 days post infection. LTC5 cells injected s.c. together with the infective promastigotes significantly enhanced subsequent lesion development (Fig. 1). Such enhancement was not evident, however, when LTC5 cells were injected i.v. or LTC5 culture supernatant was used to mix with the infecting parasites. No lesion developed from a s.c. injection of 5 × 10⁶ LTC5 cells alone. These results demonstrate that the cloned LTC5 cells were able to exert their deleterious effect when injected locally into the site of parasite inoculation at the onset of infection. The failure of i.v. transferred LTC5 cells or their supernatant to affect the lesion develop-

Table 1 Suppression of induction of DTH *in vivo* by a cloned T_s -cell line or its culture supernatant

Transferred (i.v.)	<i>L. tropica</i>		SRBC	
	DTH (10^{-2} mm)	% Suppression	DTH (10^{-2} mm)	% Suppression
LTC5 cells (10^7)	9 ± 3	<u>86.2</u>	98 ± 5	3.2
Normal T cells (10^8)	34 ± 2		101 ± 7	
LTC5 supernatant (0.5 ml)	10 ± 2	<u>80.0</u>	110 ± 8	0
Control supernatant (0.5 ml)	30 ± 3		88 ± 6	
Negative controls	5 ± 1		10 ± 2	

Spleen cells from BALB/c mice infected s.c. 69 days previously with 2×10^7 *L. tropica* promastigotes were cultured at 10^6 ml $^{-1}$ with irradiated (2,000 rad) syngeneic spleen cells (feeder cells, 10^6 ml $^{-1}$) and FFP (10^6 ml $^{-1}$) in 10 ml medium [RPMI 1640 (Flow Laboratories) supplemented with penicillin streptomycin, glutamine (2 mM), 2-mercaptoethanol (5×10^{-5} M) and fetal calf serum (FCS, 10%)] and 20% TCGF in 25 cm 2 tissue culture flasks (Falcon). After 7 days of primary culture, viable cells were isolated on Lymphocyte-M (Cedarlane) and recultured at 10^6 ml $^{-1}$ with feeder cells, FFP and TCGF as before. The culture was fed every 5–7 days with fresh medium, TCGF, FFP and feeder cells. The viable cells were purified, expanded and tested in functional assays whenever necessary. When the cell line was expanding vigorously it was cloned at 0.5 cell per well in sterile 60-well Terasaki micotest. II trays (Falcon) as follows: 3 days after the addition of fresh medium, antigen and feeder cells, viable cells were harvested on Lymphocyte-M. All dilutions were made in the bulk culture supernatant from which the viable cells were conditioned. To each micotest well were added 10^5 feeder cells and 10^5 FFP. The plates were left undisturbed for 5 days after which half of the medium (10 μ l) was replaced by fresh medium containing TCGF (20%), FFP (10^6 ml $^{-1}$) and irradiated spleen cells (10^6 ml $^{-1}$). This was repeated at 3–4 day intervals. The wells that showed positive growth (2%) were transferred to 96-well flat-bottom microtrays (Falcon) and cultured with feeder cells (10^6 per well), FFP (10^6 per well) and TCGF. The clones were gradually expanded through 24-well trays (Costar), 25–75 cm 2 tissue culture flasks (Falcon) always in the presence of antigen, irradiated spleen cells and TCGF. TCGF was prepared from rat spleen cells (2×10^7 cm $^{-2}$) pulsed for 2 h with concanavalin-A (Miles-Yeda, 20 μ g ml $^{-1}$) followed by extensive washing and further culturing at 5×10^6 ml $^{-1}$ in fresh medium for 48 h. At the end of culture, supernatant was cleared by centrifugation and filtration through Millipore and stored in aliquots at -20°C until use. One of the clones (LTC5) which showed rapid growth was tested for suppressive activity for induction of DTH to FFP. BALB/c mice were injected i.v. with 10^7 Lymphocyte-M-purified LTC5 cells, or 0.5 ml 5 days LTC5 culture supernatant, or 10^8 anti-immunoglobulin column enriched⁸ normal T cells, or 0.5 ml 5 days culture medium of feeder cells, FFP and TCGF (control culture supernatant without LTC5 cells). The supernatants were harvested by centrifugation at 2,000g. All mice except the negative controls were immunized s.c. with 4×10^7 FFP or 10^8 SRBC immediately after cell or supernatant injection. DTH was elicited in the hind footpad 7 days later with 10^7 FFP or 10^8 SRBC (in 50 μ l phosphate-buffered saline). Footpad swelling was measured 3, 24 and 48 h later with a reverse spring-loaded dial-caliper (Pocotest, Carobronze, London). DTH is expressed as 24-h footpad thickness increase in 10^{-2} mm $\pm$ s.e.m. Negative controls represent background inflammation due to the eliciting antigens alone. % Suppression = [(DTH control – DTH background) – (DTH test – DTH background)] / (DTH control – DTH background) $\times 100$. Figures underlined are statistically significant ($P < 0.005$, $n = 5$).

ment may be explained by the altered recirculatory property of the cultured cells¹² and the short half life of the culture supernatant in a chronic infection.

Cell-surface marker analysis using complement-dependent cytotoxicity demonstrated that LTC5 cells possess the Lyt-1^{+2-} , I-J^{-} phenotype consistent with a previous functional depletion study on a population of T_s cells⁹. Electron microscopy showed most of the LTC5 cells to contain an eccentrically located nucleolus and numerous electron-lucent vesicles containing granules of various stages of maturity, surrounding densely packed Golgi areas, a feature consistent with cells engaging in active secretion. Similar ultrastructure has also been reported for cloned natural killer cells and Lyt-1^{+2-} suppressor cells for antibody response to SRBC¹³. Unlike TD or TDA, LTC5 is TCGF dependent, though antigen synergizes with TCGF in promoting LTC5 proliferation. The requirement for an interleukin-2 (IL-2) signal in T-cell growth is implicit^{14,15}. The

Table 2 Suppression of specific *in vitro* proliferation of effector cells by cloned T_s cells and their culture supernatant

Cells	Antigens (c.p.m.)	
	<i>L. tropica</i> + irradiated BALB/c spleen cells	Irradiated CBA spleen cells
LTC5	375 $\pm$ 120	203 $\pm$ 90
TD	17,540 $\pm$ 340	410 $\pm$ 58
TDA	370 $\pm$ 101	10,070 $\pm$ 450
TD + LTC5	1,030 $\pm$ 110	NT
TDA + LTC5	NT	11,340 $\pm$ 321
TD + supernatant	920 $\pm$ 240	NT
TDA + supernatant	NT	9,832 $\pm$ 521

TD and TDA are two uncloned T-cell lines derived from BALB/c mice as follows: TD, lymph node cells from a BALB/c mouse, sublethally (550 rad) irradiated and infected with 2×10^7 *L. tropica* promastigotes on day –60, were cultured at 10^6 ml $^{-1}$ in culture medium (see Table 1 legend) with FFP (10^6 ml $^{-1}$) and irradiated BALB/c spleen cells (10^6 ml $^{-1}$) in 25-cm 2 tissue culture flasks (Falcon) without TCGF for the first 7 days. Thereafter the viable cells were isolated by Lymphocyte-M and recultured as above in the presence of 20% TCGF. TDA was derived from lymph node cells of a BALB/c mouse injected (i.p., day –12) with 150 mg per kg cyclophosphamide, immunized (s.c., day –10) with 10^8 irradiated (2,000 rad) CBA spleen cells and boosted (footpad, day –2) with the same antigen. The immune lymph node cells (10^6 ml $^{-1}$) were co-cultured with irradiated CBA spleen cells (10^7 ml $^{-1}$) in the absence of TCGF for the first 7 days, thereafter they were purified on Lymphocyte-M and co-cultured with irradiated CBA spleen cells and 20% TCGF. Both TD and TDA have been cultured continuously for more than 2 months before use for testing against LTC5. In this test, carried out in the absence of TCGF, LTC5, TD or TDA cells (10^4 cells per well) were placed in a 96-well flat-bottomed tray (Falcon). To these were added 0.1 ml culture medium, or 5 days LTC5 culture supernatant, or 10^4 LTC5 cells as indicated. To each well was also added either 10^5 irradiated CBA spleen cells or 10^4 FFP and 10^4 irradiated BALB/c spleen cells, the total volume being 0.2 ml per well. After 72 h, cultures were pulsed with 1 μ Ci of ^3H -TdR and cells were collected 18 h later. Incorporation of ^3H -TdR was measured by liquid scintillation spectroscopy. Results are expressed as c.p.m. $\pm$ s.e.m. of six replicates. NT, not tested.

apparent independence of TCGF of TD and TDA cells is likely to be due to the ability of these effector cells to produce endogenous IL-2. Indeed, culturing primed spleen cells initially in medium free of TCGF tends to enrich effector cells and select against T_s cells (Table 2 legend). The LTC5 clone has no detectable cytotoxic activity against infected macrophage target cells nor does it enhance anti-*L. tropica* antibody response in an adoptive transfer experiment, indicating a lack of cytotoxic or helper activities.

The cloned T_s cells are, however, antigen specific and suppress the *in vitro* proliferation of effector cells as well as the *in vivo* induction of DTH to *L. tropica*. Furthermore, they also appear to be capable of enhancing lesion development of *L. tropica*-infected BALB/c mice, suggesting a causal role in the pathogenesis of the disease. This report thus confirms at the clonal level the existence of Lyt-1^{+2-} , I-J^{-} T_s -cell populations generated during *L. tropica* infection of genetically vulnerable BALB/c mice⁹. Using the LTC5 clone, the mediation of suppressive activity through soluble suppressor factor(s) was shown, a feature not demonstrable previously in the present system using uncloned T_s -cell populations. The success here is probably due to the higher concentration of suppressor factor attainable using the cloned cells. The cloned T_s line should therefore facilitate further characterization of the suppressor factor.

So far three cloned specific T_s lines have been reported in the literature: two mouse T-cell lines suppressing the *in vitro* humoral response to SRBC¹⁶ and bovine serum albumin¹⁷ respectively, and a human T-cell line inhibiting the *in vitro* proliferation of an autologous helper-T-cell clone specific for the matrix protein of influenza virus¹⁸. The paucity of cloned specific T_s cells may reflect their intrinsic instability in long-term culture. LTC5 is functionally stable but variable in its growth rate. It is TCGF dependent, but proliferates best when antigen and feeder cells are also present in the culture. Cloning efficiency is enhanced by carrying out limiting dilution in supernatant from the bulk culture maintaining vigorously growing T_s -cell lines, rather than in fresh medium. The present paper, as far as I am aware, represents the first report on cloning of T_s cells with both *in vitro* and *in vivo* functions against a parasitic disease.

Helper T-cell clones with effector functions against *L. tropica*¹⁹ and *Listeria monocytogenes*²⁰ are already available.

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Acetylcholine release from growth cones detected with patches of acetylcholine receptor-rich membranes

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Synaptic potentials can be evoked at nerve-muscle junctions *in vitro* within minutes after an exploring growth cone contacts a receptive myotube^{1–3}. Functional transmission is also evident *in vivo* on the time scale of minutes after motor axons enter adjacent myotomes^{4,5}. The ability to release acetylcholine (ACh) may be induced in motor nerve terminals after they contact receptive target cells. Alternatively, growth cones may be capable of releasing ACh before contact. To examine the development of ACh release we have used isolated patches^{6,7} of acetylcholine receptor (AChR)-rich membrane as sensitive detectors of ACh. We report here that the growth cones of embryonic chick ciliary ganglion neurones can release ACh, even when the cells are grown in the absence of target myotubes.

We used cultured chick myotubes as the source of AChR-rich membrane, as these cells are sensitive to ACh over their entire surface⁸. Myoblasts dissociated from pectoral muscles of 11-day embryos were plated in dishes containing collagen-coated chips of glass coverslips (~2 mm × 2 mm). Neurones dissociated from 8- or 9-day-old embryonic ciliary ganglia were routinely plated in plastic dishes on a layer of lysed fibroblasts⁹. Similar results were obtained when the neurones were plated on collagen- or polylysine-coated surfaces, although neurite outgrowth was less extensive on these polymers. Because it was important to visualize the entire extent of each neurone, the cells were seeded at relatively low density; on average there was less than one neurone per field of view (530 µm across).

At the start of each experiment a single glass chip with adhering myotubes was placed in the culture dish. During experiments the growth medium was replaced with a buffered salt solution supplemented with amino acids and containing 10⁻⁶ g ml⁻¹ eserine to inhibit acetylcholinesterase. Cultures were mounted on the stage of an inverted compound microscope and observed with a ×32 phase contrast objective. Experiments were done at room temperature except where indicated. Outside-out muscle membrane patches were prepared as described

by Hammil *et al.*⁷ using borosilicate glass electrodes pulled to a tip diameter of 1–2 µm. The inside of the electrode was held at a fixed potential (usually –60 or –70 mV) and the current across the patch was monitored with a List LM-EPC5 patch-clamp amplifier.

The sensitivity of isolated patches was tested by iontophoresis of ACh from a fine-tipped electrode positioned within a few micrometres of the patch. When a brief pulse of ACh was applied, discrete channel openings were evident; see Fig. 1A(i). The typical single-channel current was ~3 pA at –70 mV. At this holding potential the apparent mean open time was ~6 ms at room temperature and 2.5 ms at 35 °C. A relatively long-duration ACh pulse applied to the same patch produced a large inward current that desensitized rapidly. In the example shown in Fig. 1A(ii), the peak inward current was >600 pA and the response declined to half the maximum within 400 ms. Judging from the amplitude of single-channel openings and the peak inward current, this patch contained at least 200 functional receptors. The number of functional receptors in patches prepared from myotubes of different platings was in the range 5–300. This important parameter is discussed further below.

Outside-out patches were sensitive enough to detect amounts of ACh that produced depolarizations similar to small synaptic potentials. In the experiment shown in Fig. 1B(i) a brief ACh pulse produced a 1-mV depolarization in an intact myotube. The input impedance of this myotube was 300 MΩ and the peak inward current underlying the depolarization was only 20 pA; Fig. 1B(ii). This is of the order of the current typically generated during evoked endplate potentials at newly formed synapses *in vitro* (L.W.R., unpublished observations). When the ACh pipette was positioned 2 µm away from an outside-out patch, an identical pulse resulted in three channel openings; see Fig. 1B(iii). The first one occurred less than 2 ms after the pulse, the second overlapped the first, and the third one occurred 60 ms later. It therefore seemed likely that a critically positioned, sensitive membrane patch might detect the release of ACh equal to or even less than that contained in a single quantum.

We studied over 150 ciliary ganglion neurones within 30 h after plating. During this time, the processes were less than 400 µm long and each cell had at least one active growth cone in view. Figure 2 shows a typical cell. In each case the patch electrode was placed within 2–4 µm of the flat part of a growth cone. Neurones were usually stimulated through an extracellular electrode sealed to the cell body by gentle suction. Rarely did we observe channel openings following a single spike, but when neurones were stimulated with short trains of pulses, channel openings were detected for about one-third of the growth cones (59 of 188). Figure 3A shows a typical example. The first opening occurred ~120 ms after the first spike, and sporadic openings were seen during the rest of the train and for several hundred milliseconds thereafter. In some cases the latency to the first channel opening was as short as 40 ms but in others it was as long as 4 s. The response following trains of impulses fatigued rapidly. In fact, in most cases only the first train resulted in detectable ACh release, even when the trains were separated by 5-min intervals. We never observed release on more than four successive trials. Channel openings were detected following single stimuli in only three cases. In the example shown in Fig. 3B, one opening was detected ~40 ms after the soma spike. A second stimulus evoked one opening and a third resulted in two openings. All of the openings appeared with approximately the same latency, indicating that they were indeed evoked responses.

The stimulus-evoked channel openings were judged to be due to the action of ACh because the amplitude and duration of the channel events were indistinguishable from those evoked by ACh. Moreover, in 10 experiments in which 10⁻⁵ M tubocurarine was added to the bath, no stimulus-evoked channel openings were detected.

We did not detect spontaneous release of ACh even though we waited at least 1 min after placement of the patch electrode near growth cones before contacting the cell body with the stimulating electrode. This result is in accord with the fact that

Fig. 1 Responses of outside-out patches of chick myotube membrane. **Ai**, A relatively brief 10-nA pulse of ACh from an iontophoretic pipette (bar below trace) gave rise to discrete inward current channel openings in a patch located 10 μm away. **ii**, A much longer 10-nA pulse produced a large inward current representing simultaneous activation of many AChR channels (note the change in scales). Membrane potential, $V_m = -60$ mV. **Bi**, A small depolarization evoked in an exact myotube by a 2.5 nA $\times$ 1 ms ACh pulse (delivered at arrowhead). V_m , -61 mV. Whole cell recording. **ii**, The current underlying the ACh response recorded under whole cell voltage clamp; V_m , -60 mV. **iii**, Same iontophoretic pulse applied to an isolated membrane patch. In this patch the peak inward current in response to a large ACh pulse was 150 pA. V_m , -60 mV. In all experiments the extracellular solution contained (in mM): NaCl 113, KCl 5, MgCl_2 0.16, MgSO_4 0.64, NaH_2PO_4 0.8, CaCl_2 3.8, HEPES 11, glucose 5 plus 1 $\mu\text{g ml}^{-1}$ eserine, 0.02% horse serum, 0.02% minimal essential medium (MEM) amino acids, 5 U ml^{-1} penicillin and 5 $\mu\text{g ml}^{-1}$ streptomycin, pH 7.3. The intracellular solution contained (in mM) KCl 140, MgCl_2 2, K_2EGTA 11, CaCl_2 1, HEPES 10, pH 7.3.

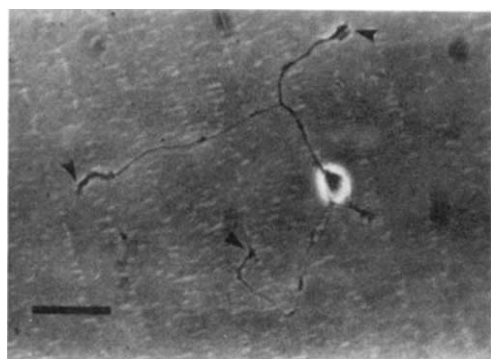
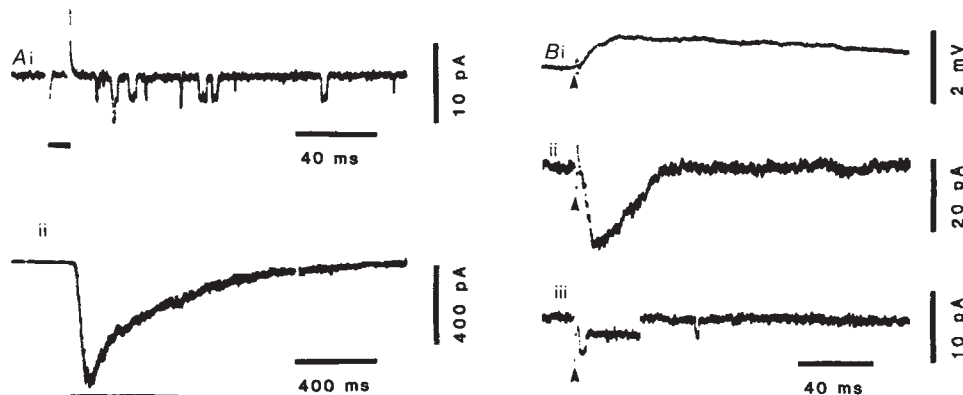


Fig. 2 Phase contrast micrograph of a cultured chick ciliary ganglion neurone 18 h after plating. Growth cones are indicated by arrowheads. Scale bar, 50 μm . Neurones were dissociated as described in ref. 9 and plated in medium containing 85% MEM, 10% heat-inactivated horse serum and 5% chick embryo extract, with penicillin and streptomycin added.

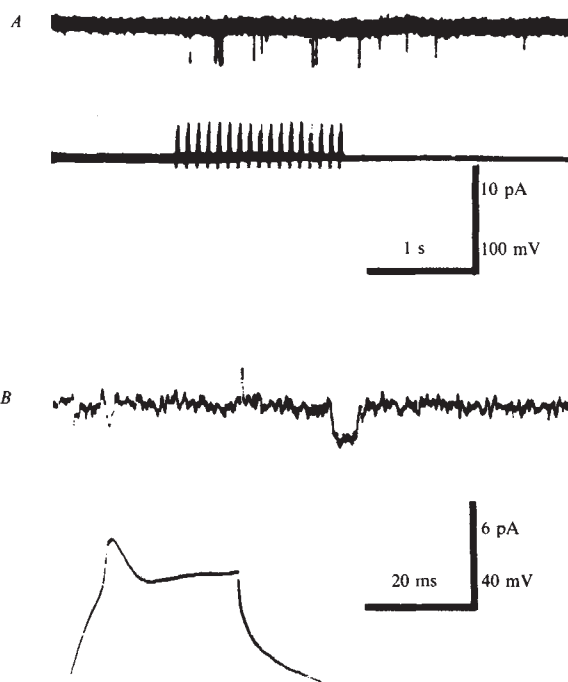


Fig. 3 Release of ACh from growth cones. **A**, A train of impulses evoked at the cell body (lower trace) resulted in the opening of inward current channels in a patch positioned at one of the growth cones. V_m , -70 mV; temperature, 35 $^{\circ}\text{C}$. This neurone was stimulated through an extracellular electrode. Action potentials were evoked by each pulse. **B**, One of three cases in which a single action potential in the presynaptic cell evoked a response. Holding potential, -60 mV. This neurone was stimulated through an intracellular electrode. The incidence and latency of responses did not appear to depend on the mode of stimulation.

we only rarely detected release following single spikes. In our culture conditions, the neurones are silent, or spike at a low frequency. In some patches there were occasional 'spontaneous' channel openings even when the patch was located high in the bath far from any neurone. The frequency of channel openings in these patches was not noticeably increased when the electrodes were placed near growth cones.

The long latency between the onset of stimulation and the first channel openings must be accounted for. In a few cases the first channel openings were detected after 40–50 ms, but more often the latency was >200 ms. The delay is not a property of the monitoring membrane patch; responses to iontophoresed ACh can begin within a few milliseconds (Fig. 1). It might be that ACh is released at some distance from the growth cone, and that the delay is due to diffusion. In fact, Johnson and Pilar¹⁰ provided evidence that ACh can be released from denervated ciliary ganglion neurone cell bodies *in vivo*.

To gain an appreciation of the magnitude and latency of the effect of ACh released from a distant source, the response of a patch to a fixed pulse of ACh was examined as a function of distance from an iontophoretic pipette (Fig. 4). When the patch was 4 μm from the source there was a large shower of channel openings at short latency that superimposed to give a relatively large inward current. At 20 μm , there were only a few openings at a considerably longer latency (first opening = 180 ms), and at 40 μm there were no detectable channel openings. This result

makes it unlikely that the ACh we detected at growth cones was released from a distant source. Most growth cones examined in these experiments were >100 μm away from the cell body, but Fig. 4 demonstrates that a distance of 40 μm is sufficient to render a relatively large pulse of ACh undetectable. If the source was even more distant the release would have to be proportionately larger. Thus, recordings from the proximal axon or cell body would be expected to show large inward currents at short latency. In fact, we did detect a few channel openings when patch electrodes were placed near some cell bodies (8 out of 40 tested), but we never recorded a large inward current. In

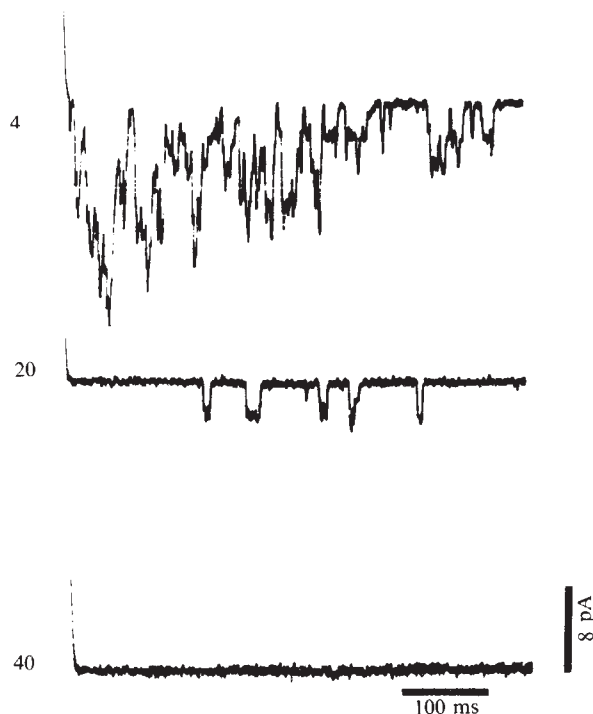


Fig. 4 ACh responses of a patch placed at different distances from the iontophoretic electrode. The interelectrode distance (in μm) is indicated to the left of each trace. The same iontophoretic pulse ($20 \text{ nA} \times 3 \text{ ms}$) was used in all cases. V_m , -100 mV .

addition, the latencies of responses recorded near cell bodies were just as long as those recorded at growth cones.

It is interesting that the first synaptic potentials evoked at newly formed nerve-muscle synapses *in vitro* sometimes have rather long latencies (up to 60 ms)³. Some of the latencies observed at growth cones fall in this range, but many responses began more than 1 s after the onset of stimulation. Thus, we tentatively conclude that the mechanism of ACh release from exploring nerve endings prior to contact is not identical to that observed at newly formed synapses. Several factors might account for the weak coupling between stimulation and secretion. It seems likely that growth cones are electrically excitable and that they contain voltage-dependent Ca^{2+} channels^{11,12}, nevertheless, the inward calcium flux might be insufficient to trigger rapid and reliable release. For example, the density of Ca^{2+} channels might be low or synaptic vesicles might be located relatively far from the sites of calcium entry. Alternatively, growth cone release might not depend on Ca^{2+} in the usual way or on synaptic vesicles. Transmitter might be stored in and released from other compartments such as the endoplasmic reticulum or the cytoplasm.

Our estimate of release from one-third of the neurones represents a minimum value. The number of functional receptors in myotube membrane patches varied widely. Among those of known sensitivity, we never detected release with a patch that exhibited a peak inward ACh current of $<60 \text{ pA}$ (~ 20 channels). It seems likely, therefore, that at least some of the patches were insufficiently sensitive to detect the amount of ACh released at growth cones. This requirement for high receptor density is the reason that we did not use patches from ciliary ganglion cells, even though these neurones are sensitive to ACh. Given the variability in the sensitivity of patches that we used, it remains possible that every growth cone can release transmitter. We also cannot exclude the possibility that growth cones release transmitter spontaneously below the limits of our most sensitive detector. In any case, one must consider the possible functional significance of pre-contact release of neurotransmitter, and perhaps of other substances also.

We have focused on release from cholinergic neurones because of our interest in nerve-muscle synapse formation. However, we suspect that the outside-out membrane patch bioassay described here will be useful in studies of other types of developing and mature synapses. One simply needs a membrane source with a sufficiently high receptor density, and access to putative sites of transmitter release.

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Spontaneous release of transmitter from growth cones of embryonic neurones

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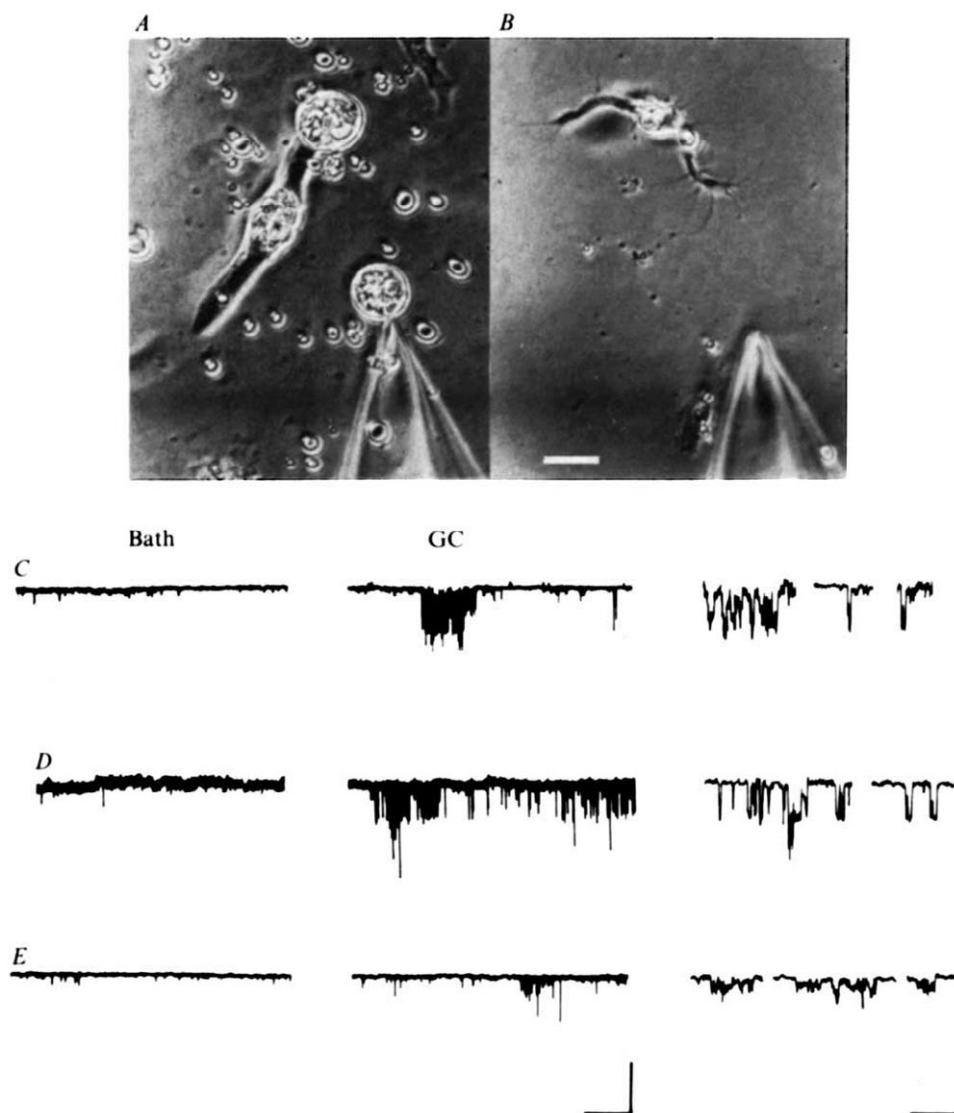
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A nerve process grows by inserting new membrane material at its advancing tip, the growth cone¹. In embryonic cell culture and in embryos of *Xenopus laevis*, many growth cones establish functional synaptic transmission within minutes after contact with muscle cells^{2,3}. The rapidity of synapse formation suggests that the growth cone may have already acquired the appropriate neurotransmitter and the machinery for transmitter release before encountering the target cell. Here, we have used a patch of outside-out embryonic muscle membrane formed with gigaohm seal at the tip of a micropipette as an extracellular probe for the presence of channel-activating substances near the growth cones of the isolated *Xenopus* embryonic neurones in culture. We report that single-channel activity resembling that of muscle acetylcholine receptor channels was induced when the probe was positioned near the growth cones of 50% of the neurones, suggesting the spontaneous release of acetylcholine (ACh) from these growth cones. The release of material from growth cones may occur as a consequence of the incorporation of new membrane during neurite extension; it may also have a role in the interaction between the growth cone and its immediate environment.

The 'excised patch' technique⁴ provides a means of detecting minute amounts of channel-inducing substances. When a pipette containing an outside-out patch of *Xenopus* muscle membrane (the probe) was positioned near the growing cone of a neurite, a marked increase in single-channel activity was frequently recorded. Figure 1 shows three examples of paired recordings at positions far away (Bath) and either $25 \mu\text{m}$ or $3 \mu\text{m}$ from the growth cones of three different neurites. The increased channel activity near the growth cone usually occurred in 'clusters' of single or multiple channel events separated by long periods of quiescence. This activity was predominantly due to activation of channels resembling ACh receptor (AChR) channels in the probe: at 22°C , these channels had a mean conductance of $35\text{--}45 \text{ pS}$, a mean channel open time (burst duration)⁵

Fig. 1 Presence of channel-inducing substances near isolated growth cones detected with an ultra-sensitive probe. *A* and *B*, preparation of the probe: *A*, a glass micropipette was pressed against the surface of embryonic *Xenopus* muscle membrane to form a GΩ seal. Suction applied to the pipette broke the membrane patch under the pipette and allowed access to the cell interior (resting potential -50 to -70 mV). *B*, The micropipette was detached from the muscle cell. An outside-out patch of muscle membrane forms at the tip of the pipette spontaneously during detachment¹⁴. The excised patch, containing muscle membrane receptors, was voltage-clamped at the whole cell resting potential, and placed near the growth cone of a neurite. Scale bar, $30\ \mu\text{m}$. *C* and *D*, Tracings of single-channel current recordings (inward current downward, filtered at 150 Hz) at positions hundreds of μm away (Bath) and $25\ \mu\text{m}$ (*C*) or $3\ \mu\text{m}$ (*D*) away from the centre of the growth cones (GC) of two different neurites. Note the increase in single-channel activity for growth cone recordings and the clustered appearance of channel events. Representative single-channel currents recorded at the growth cone are shown at higher time resolution (filtered at 1 kHz) at the right of each growth cone recording. These events resemble those of ACh-activated channels in muscle membrane (see text). *E*, Similar recording to that shown in *D*, except that the culture was pre-incubated with α -BGT ($20\ \mu\text{g ml}^{-1}$) for 20 min before the membrane patch was prepared. No increase in ACh channel-like activity was observed near the growth cone, although there was an increase in activity of small (1 pA) channels. Scale bars for bath and GC traces, 4.0 pA and 10 s; for higher resolution traces, 4.0 pA and 50 ms.

Methods: *Xenopus* nerve-muscle co-culture was prepared by a procedure similar to that reported previously¹³. A glass micropipette of tip opening $1\ \mu\text{m}$ was prepared by the standard procedure of two-stage pulling, Sylgard coating, and fire polishing¹⁵. The pipette was filled with an internal solution of (in mM): 0.67 NaCl, 105 KCl, 1.3 MgSO_4 , 1 CaCl, 11 EGTA, and 5 HEPES-KOH buffer at pH 7.8 . The external solution consisted of (in mM): 116 NaCl, 0.67 KCl, 0.42 CaNO_3 , 1.3 MgSO_4 and 5 HEPES-NaOH buffer at pH 7.8 . Temperature for all experiments, $22 \pm 1^\circ\text{C}$.



of 3 ms, and a reversal potential between -10 and -15 mV, properties identical to those of the ACh-activated channels known to be present on these embryonic *Xenopus* muscle cells during the first 2 days in culture^{6,14}. Furthermore, in five separate experiments the muscle membrane patch was prepared after pre-incubation of the muscle cell with α -bungarotoxin (α -BGT; Sigma), a snake toxin that irreversibly blocks ACh activation of the AChR channel in these muscle membranes; we observed no significant difference in AChR channel-like activity between the background and growth cone recordings. Taken together, these results suggest that ACh was released by some of the growth cones and that it induced single-channel currents in the probe.

The *Xenopus* nerve-muscle culture used in the present study consists of mixed neuronal types. Previous studies of synaptic formation in this culture suggested that $\sim 60\%$ of the neurones are cholinergic and capable of forming functional synapses with co-cultured muscle cells (Y. Kidokoro, personal communication). Of a total of 35 growth cones studied, increased AChR channel-like activity was observed in 18 growth cones ($\sim 50\%$). For these, presumably cholinergic, growth cones, the average

opening rate of AChR channel-like events was 25 per 10 s (range 1.7 – 77 , $n = 6$) for a probe positioned 3 – $5\ \mu\text{m}$ from the growth cone. Most channel events appeared in clusters, at an average frequency of ~ 2 per min. Channel opening rates decreased as the probe was positioned further away from the growth cone. Figure 2 is a plot of the average channel opening rate for AChR channel-like events at various distances from the growth cones. Increased channel activity was clearly detected at distances of 25 – $50\ \mu\text{m}$, where contact of the probe with the growth cone was unlikely to occur. This suggests that the increase in channel activity resulted from the presence of ACh which was spontaneously released from the neurone. The release was apparently restricted to the growth cone: in three separate experiments, the probe was positioned near the neuronal soma and along the neurites at least $50\ \mu\text{m}$ away from growth cones exhibiting release, and no increase in channel activity above the background level was observed.

High levels of release of ACh-like material were most easily detected when the pipette was positioned 3 – $5\ \mu\text{m}$ from the centre of the 'palm' of the growth cone. For these recordings, the probe was positioned at a 45° (azimuthal) angle above the

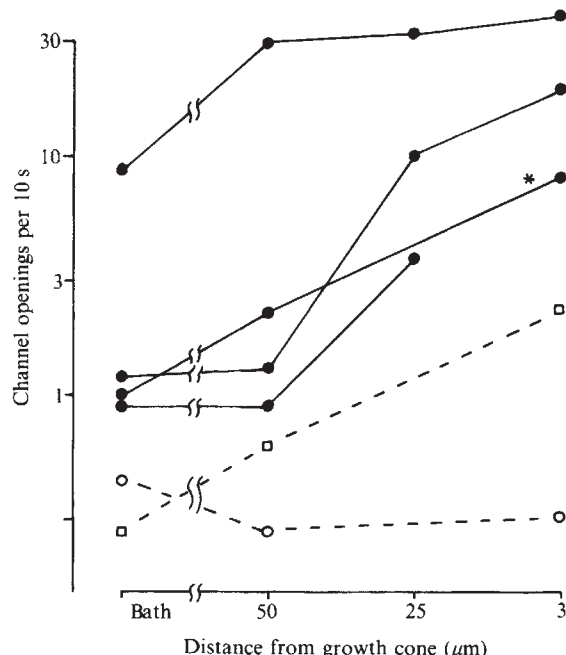


Fig. 2 Changes in channel opening frequency with the distance of the probe from the growth cones. Results are from four separate growth cones. Data points connected by each curve depict channel opening frequency at various distances from the growth cone during a sequential recording (in the following order): hundreds of μm away (Bath), and at 50, 25 and 3 μm from the growth cones. Total sample time for each point was at least 90 s. ●, Events resembling those of ACh-activated channels (criterion: single-channel current within range 1.5–3.0 pA, no more than five rapid closures per opening burst). Open symbols, Opening frequency of two other channel types recorded near the same growth cone as the marked (*) curve for the AChR channel-like activity. Open circles represent the frequency of a small (1.0 pA) channel of short open time (<20 ms) that exhibits a nearly constant opening rate at all distances. Open squares represent a rapidly 'flickering', small (1.0 pA) channel of long open time (>20 ms) that exhibits increased opening frequency on approach to the growth cone. Activity of the latter two unidentified channels and the presumptive AChR channels accounts for all the single-channel events observed near this particular growth cone. This study suggests that the growth cone releases ACh spontaneously in the absence of possible probe contact, and the concentration of the released ACh decreases at positions progressively further away from the growth cone. The AChR channel activity in the bath may vary by 10-fold. Since the slide containing the cells is washed at the start of the experiment, and some recordings are made as long as 2 h later, variations in background levels of ACh may be expected in the culture, which itself contains a variable density of neuronal cells.

growth cone and the substratum surface to avoid contact of the probe with the growth cone. Although there was no visible contact, we cannot exclude the possibility that the probe had touched some undiscernibly fine filopodia which arose from the substratum. Thus, it is possible that channel events observed at this close position were due in part to probe-induced release of substance. Contact-induced release, if it occurred at all, must result from specific interaction of the muscle membrane with the growth cone membrane, as gentle but visible contact using a separate fine glass rod at the recorded growth cone did not cause any increase in channel activity in the adjacent probe. Moreover, the increased channel activity recorded near the growth cone cannot be attributed to a contact-induced electrical artefact in the probe: in three separate experiments, the probe was positioned to touch a muscle cell or a neuronal soma in the same culture, and no effect on channel activity in the probe was observed within 5 min of continuous recording.

Recordings near the growth cone occasionally showed multi-

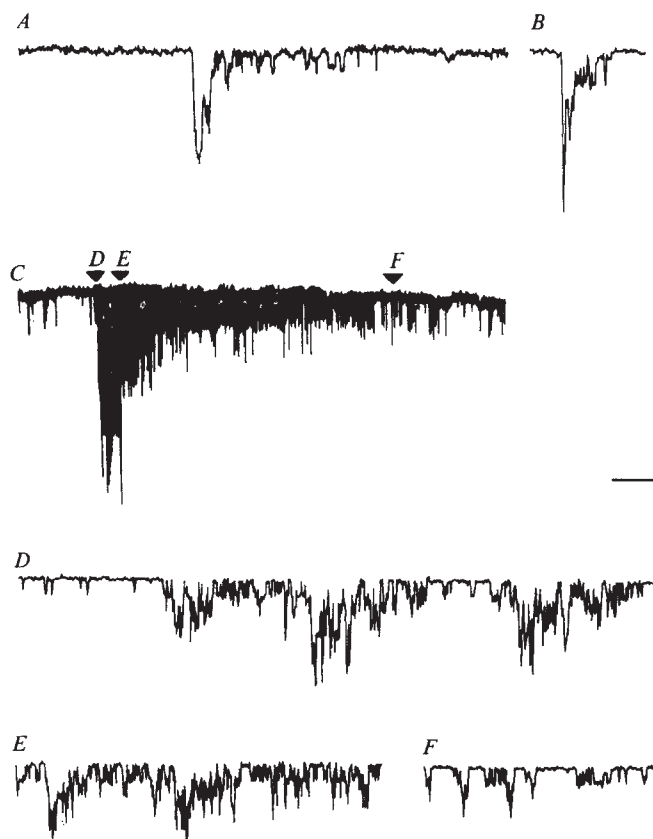


Fig. 3 'Staircase' channel activity recorded near the growth cone. *A, B*, Two examples of multiple-channel events recorded near two different growth cones that showed low-level activity. Note the rapid opening and slower decline of multiple open channels. *C*, A recording of high-level activity from a third growth cone shown on a compressed time scale. The probe was positioned 3 μm away and above the growth cone in all three cases. The high-level activity, preceded by 80 s of low-level activity, lasted for ~2 min. Channel currents are also shown in higher time resolution and lower gain for three periods during the high-level activity: at the onset (*D*), 5 s after the onset (*E*), and near the end (*F*) of the activity, as marked by arrows in *C*. Note that the high-level activity was in fact composed of a sequence of 'staircases', each with a pattern similar to that shown in *A* and *B*. Between the consecutive 'staircases' and after the last 'staircase', the current trace returned to the same baseline as that at the onset of the activity, indicating no significant change in the seal resistance at the probe. These staircase patterns seem to reflect pulsatile release of ACh from the growth cones. Scale bars: *A*, 4 pA and 50 ms; *B*, 4 pA and 25 ms; *C*, 4 pA and 10 s; *D–F*, 8 pA and 50 ms.

ple channel openings in a 'staircase' pattern, which was characterized by an initial rapid opening of two or more channels followed by a decay to single-channel openings and eventually to no activity. Figure 3*A, B* shows two isolated cases of 'staircase' patterns recorded during low-level activity near two different growth cones. Prolonged, high-level activity (Fig. 3*C*) was found to consist of a sequence of multiple channel openings of the staircase pattern. Figure 3*D–F* shows the channel opening pattern at the onset, 5 s after onset, and near the end of the high-level activity, respectively. The average duration of the staircase pattern was 138 ms, and the recording during the first 5 s of high-level activity consists of 12 staircases that could be clearly discerned. The maximum number of simultaneous channel openings was five at the onset, and progressively declined to one near the end of the discharge. The decline of the maximum number of channels available may reflect either channel desensitization in response to the released material, or a decline in the amount of material released with time, or both factors.

Ultrastructural studies⁷⁻⁹ on isolated growth cones or newly formed nerve-muscle contacts have shown that the growth cones contain vesicles of 50 nm diameter that resemble synaptic vesicles at mature neuromuscular junctions. Vesicles containing noradrenaline have been found in the growth cones of cultured rat sympathetic neurones⁹. There has also been evidence of cytoplasmic ACh in mammalian nerve terminals¹⁰. As the growth cone presumably undergoes constant exocytotic activity as part of the process of new membrane addition, the release of ACh-like material observed in the present study may reflect neurite growth whereby the ACh-containing 'presumptive synaptic vesicles' were indiscriminately fused with the growth cone membrane together with other membrane precursor vesicles. Alternatively, cytoplasmic ACh may 'leak out' during the fusion of membrane precursor vesicles with the plasma membrane of the growth cone. Our finding that activated channel openings were clustered into groups is consistent with the notion of pulsatile events of membrane incorporation or vesicular release of ACh. Computer modelling of channel openings in a patch of muscle membrane in response to a wave of ACh molecules diffusing from a nearby point source produces 'staircase' patterns of channel openings (S.H.Y., unpublished data) similar to those observed near the growth cone. Thus, it seems that the spontaneous release of ACh from the growth cone occurs in a pulsatile fashion, with simultaneous discharge of many ACh molecules within each pulse. Such release might be detected as 'staircase' multiple-channel events if the probe is near the release site and the concentration of ACh is high, and as clusters of single-channel openings if the probe is located some distance away from the release site. The pulsatile release of ACh may be related to spontaneous action potentials in these neurones which occur at about the same frequency² as that of the cluster events. Release of ACh from the growth cones of chick ciliary ganglion neurones in response to electrical stimulation of the neurone has recently been observed by a technique similar to that reported here¹¹.

Finally, we observed at least one other channel type (of a much lower frequency) which appeared when the probe was near the growth cone (see also Fig. 2). This channel has a current size about one-half that of ACh channels (at membrane potential -50 to -70 mV), opens for much longer periods of time (0.1–1.0 s), exhibits many short closures ('flickers') during an open burst, and has a reversal potential of $\sim +10$ mV. The identity of this channel as well as the channel-inducing substance remains to be elucidated.

We have illustrated the use of an excised patch of excitable membrane as a sensitive molecular probe for a spatiotemporal mapping of channel-inducing substances. We have found that some embryonic neurones spontaneously release ACh-like molecules and perhaps other channel-inducing substances from the growth cone. This release of material may be important for the interaction of the growth cone with its environment and with its target cell during the initial phases of synaptogenesis¹².

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Failure of phosphatidic acid to translocate Ca^{2+} across phosphatidylcholine membranes

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The putative role of phosphatidic acid (PA) as a Ca^{2+} ionophore has offered an attractive explanation for the relationship between Ca^{2+} influx and the turnover of phosphoinositides in stimulated cells¹. The ionophoretic properties of PA are evident in its ability to translocate Ca^{2+} across a layer of organic solvent². When added exogenously to some cells, PA produces a physiological response^{3–6} and in neuroblastoma cells stimulation of Ca^{2+} uptake can also be detected⁶. It was later shown that low levels of PA added exogenously to, or incorporated endogenously in liposomes, increase their permeability to Ca^{2+} (refs 7, 8), indicating a direct effect of PA on lipid bilayer properties. We now report, however, that we have not been able to demonstrate facilitation by PA of Ca^{2+} fluxes across liposomal membranes. Ascribing such a role to PA does not seem compatible with known features of biological membranes or the properties of ionophores known to translocate ions across membranes.

When egg yolk PA was incorporated into liposomes at concentrations ranging from 1.5 to 10 mol% or added exogenously in concentrations of 10–60 μM , no increase in Ca^{2+} permeability was detected (Fig. 1). When the time of incubation was extended

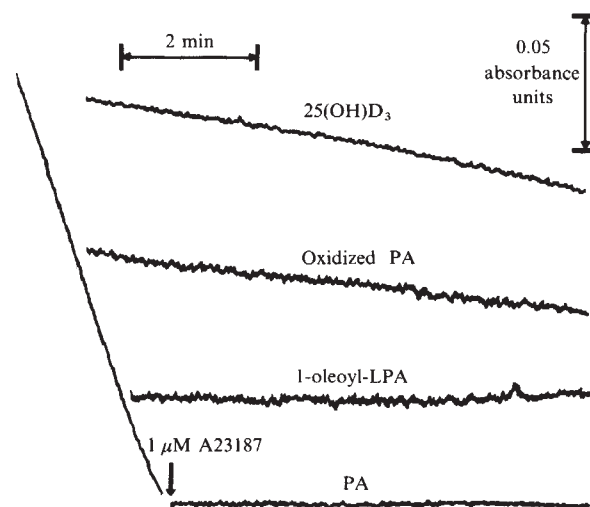


Fig. 1 Permeability of liposomes to Ca^{2+} . Sized multilamellar vesicles were prepared by extrusion of lipids through a $0.4\text{-}\mu\text{m}$ polycarbonate filter¹⁸, after dispersion in a solution containing 3.1 mM arsenazo III, 0.145 M KCl, 0.1 M Tris-HCl, pH 7.45 (ref. 19). The capture volume, 4.3 ± 0.4 litres per mol lipid was similar to that reported by Olson *et al.*¹⁸. Vesicles consisted of 70 mol% PC and either contained 10 mol% PA or lysophosphatidic acid (LPA), 10 mol% dicetyl phosphate and 10 mol% cholesterol, or they contained 10 mol% 25(OH) D_3 and 20 mol% dicetyl phosphate. Tracings of the change in arsenazo III absorption at 650 nm are shown within 20 s after the addition of 1 mM CaCl_2 . Exogenous lipids and A23187 were added from stock solutions in dimethyl sulphoxide. Using a molar extinction coefficient of $1.89 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$, calculated for the Ca-arsenazo III complex in these incubation conditions, the uptake rates measured by the method of Weissmann *et al.*¹⁹ were 0.31 mmol per mol lipid per min for the oxidized PA from P-L Biochemicals, 1.10 mmol per mol lipid per min for the 25(OH) D_3 , and 9.38 mmol per mol lipid per min for the ionophore.

to 48 h, the liposomes containing 10 mol% PA still had not taken up a detectable amount of Ca^{2+} . The Ca^{2+} ionophore, A23187, when added exogenously at a concentration of 1 μM , induced a rapid increase in arsenazo III absorption at 650 nm, demonstrating that the experimental system was able to detect changes in membrane permeability to Ca^{2+} (Fig. 1). The ionophore was permselective in translocating divalent cations in the order $\text{Ca}^{2+} \approx \text{Mg}^{2+} > \text{Ba}^{2+} \approx \text{Sr}^{2+}$. Furthermore, the incorporation of 10 mol% 25-hydroxycholecalciferol (25-(OH) D_3), a sterol that we have found to increase membrane permeability to Ca^{2+} (unpublished results), effectively enhanced Ca^{2+} influx, confirming the system's ability to respond to membrane changes (Fig. 1). Egg yolk PA obtained from two commercial sources, Sigma and Gibco, were equally ineffective. These lipids migrated as a single spot on TLC in two different solvent systems: chloroform/methanol/25% NH_4OH , 65:30:3 (v/v); and diethyl ether/ethanol/formic acid/HCl, 150:1:2:0.5 (v/v). The egg yolk PA from both sources contained a fatty acid profile similar to that reported for the parent phosphatidylcholine (PC)⁹ as measured by the relative retention time of methyl esters on a 60-m capillary SP2340 GLC column. Palmitic (31%), stearic (13%), oleic (28%) and linoleic (18%) acids were the major fatty acids. The generation of 6.7 mol% PA from PC *in situ* in liposomes consisting of PC/dicetyl phosphate/cholesterol, 70:20:10 (mol%), by the addition of 5 U ml^{-1} of cabbage phospholipase D (ref. 10) for 24 h at room temperature, did not affect Ca^{2+} permeability. Unsized multilamellar vesicles and large unilamellar vesicles prepared by reverse-phase evaporation¹¹ were also not permeable to Ca^{2+} when they contained 10 mol% PA. That PA did not affect permeability was also independently confirmed by using $^{45}\text{Ca}^{2+}$ and a dialysis sac method. The above results indicate that the inability of PA to influence membrane permeability is independent of the source of PA, the type and size of the liposomes, and the method used to measure permeability.

To explain the earlier reports^{7,8} of the Ca^{2+} -translocating ability of PA we considered whether the PA used was either contaminated by a small amount of lipid with potent ionophoretic properties or whether the PA used was structurally modified, by oxidation of unsaturated fatty acids for instance. Benton *et al.*¹² reported that lysophosphatidic acid was a common contaminant of PA preparations and that it was able to stimulate platelets, whereas PA was ineffective. It has been suggested that lysophosphatidic acid has Ca^{2+} ionophoretic properties^{13,14}. Neither 1-oleoyl- nor 1-palmitoyl-lysophosphatidic acid (Serdary, London, Ontario) were effective in translocating Ca^{2+} across PC membranes when pre-incorporated at 10 mol% (Fig. 1).

Egg yolk PA from P-L Biochemicals was found to translocate Ca^{2+} (Fig. 1). The unsaturated fatty acid content of this preparation was substantially reduced compared with the PA from the other two sources, with linoleic acid accounting for 5% of the fatty acids and oleic acid 16%. Whereas the PA from the other two companies contained an arachidonic acid content of 2–4%, this preparation contained none. Its malonaldehyde content, a measure of the extent of fatty acid oxidation, was 4.5 mmol per mol PA¹⁵. The low unsaturated fatty acid content and high malonaldehyde content of this preparation are consistent with substantial fatty acid oxidation having occurred. It has been reported that oxidized preparations of fatty acids facilitate the movement of Ca^{2+} across liposome membranes⁷, suggesting that oxidation of fatty acids in this PA preparation was responsible for the observed Ca^{2+} translocation. The malonaldehyde content of the PA preparations not supporting Ca^{2+} translocation was ~ 1 mmol per mol PA. Storage of these preparations in suboptimal conditions increased their malonaldehyde content, and they became active in translocating Ca^{2+} .

PA is structurally dissimilar to known cationic ionophores and the main mechanism proposed for its translocation of Ca^{2+} is through the formation of non-bilayer phases (inverted vesicles) in membranes^{3,6}. Such a mechanism is incompatible with the low concentration of PA in membranes, the transpositional

location of the PA synthesized and the Ca^{2+} to be translocated, and evidence that Ca^{2+} induces PA vesicles to form lamellar aggregates, shown by X-ray diffraction and freeze-fracture analyses^{16,17}. The inability of PA to translocate Ca^{2+} across PC membranes suggests that an alternative explanation should be sought for the relationship between Ca^{2+} influx and phosphoinositide turnover during stimulus-response coupling.

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c-Ha-ras1 is not deleted in aniridia-Wilms' tumour association

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Non-random tumour-specific chromosomal abnormalities have been observed in cells of many different human tumours. In Wilms' tumour (WT) and retinoblastoma, a chromosomal deletion occurs germinally or somatically^{1–4} and has been considered an important step in tumour development. One class of potential cellular transforming genes comprises the cellular homologues of the transforming genes of highly oncogenic retroviruses. A remarkable concordance between the chromosomal location of human cellular oncogenes and the breakpoints involved in acquired chromosomal translocations is becoming apparent in various cancers: the oncogenes c-mos, c-myc and c-abl are located at the breakpoints that occur in acute myeloblastic leukaemia, Burkitt's lymphoma and chronic myelocytic leukaemia respectively⁵. Thus when the oncogene c-Ha-ras1 was localized to the short arm of human chromosome 11 (refs 6–8; region 11p11 → p15 and not 11p13 as stated in ref. 5), it was proposed as a possible aetiological agent in the aniridia-WT association (AWTA) that results from a deletion of 11p13 (although a transforming gene recently isolated from a WT cell

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line (G401) was shown not to be homologous to either *c-Ha-ras* or *c-Ki-ras*⁹). We have now looked for deletion or rearrangement of *c-Ha-ras1* in the DNA from four subjects with *del*(11p13)-associated predisposition to Wilms' tumour, aniridia, genitourinary abnormalities and mental retardation. We report here that in no case is *c-Ha-ras1* deleted, and we have further refined its location to 11p15.1 → 11p15.5. On the basis of enzyme studies and direct gene dosage determination for *c-Ha-ras1* and β -globin in neoplastic and non-neoplastic tissues from one patient, we conclude that deletion of the normal counterpart of 11p cannot account for the development of the tumour.

DNA was purified from fibroblasts and Epstein-Barr virus (EBV)-established lymphoblastoid cell lines from four patients with *del*(11p13)-associated AWTAs and from the father and mother of case 1 (Table 1). After cleavage with the restriction endonuclease *Bam*HI, the DNA samples were transferred to nitrocellulose filters by the method of Southern¹⁰. The gene copy number for *c-Ha-ras1* was assessed by hybridization of *Bam*HI-cleaved blotted DNA to a mixture of ³²P-labelled *c-Ha-ras1* and $\alpha 1(I)$ procollagen cDNA used as an internal nonsynthetic control as previously described¹¹.

*Bam*HI-cleaved DNA from the four patients and normal controls contained one or two *c-Ha-ras1*-related fragments (Fig. 1). These findings are in good agreement with the already established restriction-fragment length polymorphism near the transforming gene¹². The relative hybridization intensities of the homologous 6.6- and 7.6-kilobase (kb) *Bam*HI *c-Ha-ras1* fragments were roughly identical and the sum of both peak areas had the same value as the area under the 6.6-kb fragment found in patients 3 and 4 (data not shown). Comparison of the relative hybridization intensities for *c-Ha-ras1* homologous *Bam*HI DNA fragments, using the 5-kb $\alpha 1(I)$ procollagen fragment as internal control, strongly suggested that patients 1 and 2 had both inherited two different alleles of 6.6 and 7.6 kb

for *c-Ha-ras1*, and that patients 3 and 4 had inherited two identical alleles of 6.6 kb. Analysis of the *Bam*HI restriction pattern for *c-Ha-ras1* in the parents of case 1 were consistent with simple mendelian inheritance (Fig. 2). These data preclude the deletion of *c-Ha-ras1* in these four patients and indicate that the normal cellular homologue of *c-Ha-ras1* does not map to 11p13.

To determine the exact site of breakpoints leading to the deletions in the four cases studied, we evaluated the gene copy number for other genes that map to 11p: catalase (*CAT*), 11p13

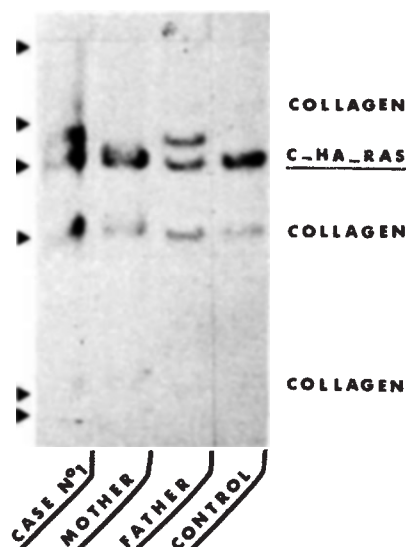


Fig. 2 Gene copy number for *c-Ha-ras1* in DNA from patient 1, his father and mother, and a normal control. For methods and symbols, see Fig. 1 legend.

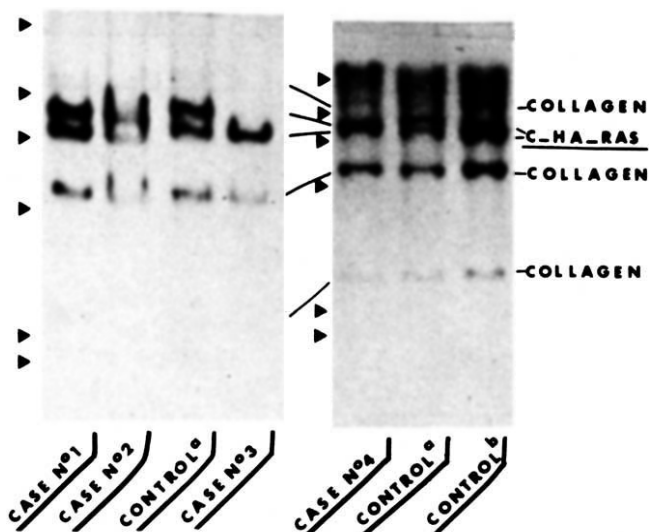


Fig. 1 Gene copy number for *c-Ha-ras1* in DNA from the four patients described in Table 1. DNA from EBV-established lymphoblastoid cell lines from patients 1, 3 and 4 and from fibroblasts (patient 2) was prepared as described previously²². Samples (10 μ g) were digested with *Bam*HI endonuclease, electrophoresed in 0.8% agarose gel and transferred to nitrocellulose filters¹⁰. The filters were hybridized in presence of 10% dextran sulphate and 50% formamide²³ to a mixture of ³²P-labelled *c-Ha-ras1* and ³²P-labelled $\alpha 1(I)$ procollagen cDNA used as an internal nonsynthetic control. The *c-Ha-ras1* probe is the pBR322 plasmid ligated to a 2.9-kb *Sac*I fragment containing all the *c-Ha-ras1* coding sequences²⁴. The $\alpha 1(I)$ procollagen probe is a cDNA probe coding for $\alpha 1(I)$ procollagen and cloned in pBR322 (ref. 25). $\blacktriangleright$, *Hind*III-digested λ DNA fragments of 23, 9.4, 6.5, 4.4, 2.3 and 2.0 kb.

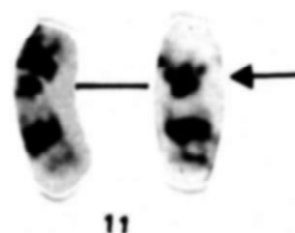


Fig. 3 Patient 4: chromosome pair 11 from GTG-stained lymphocytes.

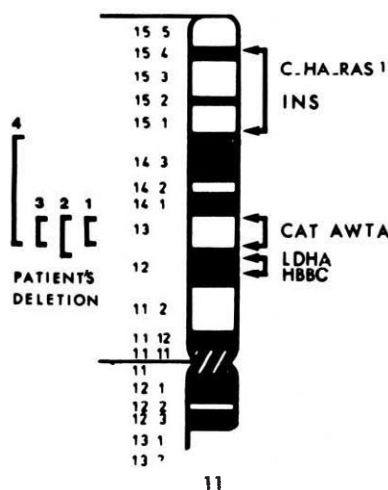


Fig. 4 Chromosome 11 short arm.

Table 1 Summary of the patients studied

Case	Age	Karyotype	Clinical features	Cells and tissues investigated
1 Proband	13 months	46XY, del 11(p13p14)	Aniridia Nephroblastoma Mental retardation	RBC, L Tumour biopsy samples: 1001 WT tumour 2001 WT tumour 3001 WT tumour necrotic sample Normal kidney biopsy sample (4001 K)
Father		46XY		RBC, L
Mother		46XX		RBC, L
2 (ref. 13)	2 yr	46XY, t(4; 7; 5) del 11(p13p14)	Aniridia Gonadoblastoma Mental retardation Ambiguous genitalia	RBC, WBC, F
3	15 days	46XY, del 11(p12p14)	Aniridia Mental retardation Ambiguous genitalia	RBC, L
4 (ref. 21)	2 yr	46XY, del 11(p13p15.1)	Aniridia Cardiomyopathy	RBC, WBC, L

RBC, red blood cell; WBC, white blood cell; L, EBV-established lymphoblastoid cell line; F, fibroblasts.

(ref. 13); lactic dehydrogenase A (*LDHA*), 11p1205→1208 (ref. 14); and β -globin (*HBBC*), 11p203→11p1205 (ref. 15). Enzymatic determinations were carried out for *CAT* and *LDHA*¹⁶ (Table 2). Direct gene-dosage determination was performed for *HBBC* using a β -globin genomic probe¹¹ and demonstrated the presence of both copies for this gene (data not shown). These experiments confirmed cytogenetic data, that is del(11p13) as indicated by the half-normal catalase levels, and established the proximal breakpoints as being more distal than the loci for *LDHA* and *HBBC*. The distal breakpoint in 11p15.1 in patient 4 was deduced from cytogenetic analyses (Fig. 3).

Recent family studies¹⁷ and somatic cell hybrids⁶⁻⁸ suggest that c-Ha-ras1 is distal to *HBBC*, so taken together with our data c-Ha-ras1 is probably located at 11p15.1→11p15.5 (Fig.

4). The gene order on 11p is: centromere-*HBBC*-*LDHA*-*CAT*, *AWTA*-*INS*-c-Ha-ras1 (refs 17, 18). It would therefore be of interest to look for a possible rearrangement of c-Ha-ras1 in relation to the t(3; 11)(p13 or 4; p15) described in a family with a renal cell carcinoma¹⁹.

Recently Benedict *et al.*²⁰ proposed that "suppression of tumorigenesis is the normal function of the diploid retinoblastoma gene". They observed that two distinct chromosomal mechanisms resulting in loss of function of region 13q14, as evidenced by no detectable esterase D activity in the tumour cells, were associated with the appearance of bilateral retinoblastoma. To test whether a similar mechanism can account for tumour development in patient 1, enzymatic and DNA analyses were carried out on two different samples (1001 WT and 2001 WT) from the histologically proven tumour, on a necrotic sample

Table 2 Enzymatic activities in cells from patient 1, his parents and patients 2, 3, 4

Case	Tissue investigated	CAT	LDHA	Enzymatic activities (IU)				Compensated ratio	
				PHI	ENO	PK	AK	CAT	LDHA
1*									
Proband	RBC	4.6	290	123	10	26	241	0.27	1.27
Father	RBC	13.5	264	92	8.3	17	231		
Mother	RBC	11.4	208	81	8.0	10	220		
1*									
Proband	L	0.0063	2.38	1.38	0.214	1.50	0.058	0.62	0.93
Mother	L	0.0116	2.92	1.58	0.217	1.80	0.074		
1*									
Proband	1001 WT	0.015	0.280	0.213	0.033	0.287	0.013		
	2001 WT	0.020	0.289	0.326	0.042	0.147	—		
	3001 WT	0.119	0.199	0.197	0.016	0.043	—		
	4001 K	0.144	2.970	2.370	7.89	0.537	0.051		
2†	RBC							0.28	1.03
	WBC							0.51	1.00
3*	RBC	6.4	220	88	10	24	—	0.39	1.02
	L	0.0062	2.50	1.56	0.207	1.56	0.073	0.65	0.92
4†	RBC							0.45	Normal
	WBC							0.47	

Enzyme assays were performed as described previously¹⁶. Enzymatic activities are expressed in IU per g haemoglobin in RBC and in IU per mg protein in the other cells. The ratio propositus/control is expressed as a compensated ratio for CAT and LDHA; to compensate for nonspecific enzymatic variations a mean ratio propositus/control was obtained for enzymes not encoded by chromosome 11: PHI, phosphohexose-isomerase; ENO, enolase; PK, pyruvate kinase; AK, adenylate kinase; and the individual ratios propositus/control for CAT and for LDHA were further divided by this mean ratio (compensated ratio). This method of calculation could not be applied to the tumour because of the large discrepancy between neoplastic and normal tissue.

* These patients will be published elsewhere.

† This patient's results have already been reported^{13,21}.

(3001 WT) and on an adjacent normal area of the kidney (4001 K) (Table 1). Dramatic enzymatic variations were found in the tumour samples (Table 2). These variations cannot be simply explained especially as enzymatic activities in the necrotic sample vary in a different way. However, they suggest that enzyme determination may not be a reliable test in tumour cells, since loss of enzymatic activity does not necessarily reflect a total loss of genetic material.

Restriction enzyme analysis on DNA from tissues and cell lines tested showed that the β -globin and c-Ha-ras1 pattern was normal (data not shown). Cytogenetic data from the tumour were not conclusive. Although we cannot exclude, in the tumour cells, a somatic deletion on the normal chromosome 11 not including *HBBC* and c-Ha-ras1, our data do not support a suppressor role for 11p13 in tumorigenesis.

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The c-Ha-ras1, insulin and β -globin loci map outside the deletion associated with aniridia-Wilms' tumour

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The localization of protooncogenes on human chromosomes may coincide with chromosome breakpoints of consistent translocations in leukaemias or lymphomas, suggesting a direct involvement of oncogenes in carcinogenesis^{1,2}. For example, in Burkitt's lymphoma consistent translocations may be associated with rearrangements of *c-myc*^{3,4}. Our assignment of the c-Ha-ras1 oncogene to chromosome 11, precisely to region 11p11→p15 (ref. 5; not 11p13 as stated in ref. 6), has raised the possibility that this oncogene might have a role in the predisposition to nephroblastoma (Wilms' tumour, WT) seen in the aniridia-WT association (AWTA)⁷ that is frequently caused by an interstitial deletion of band 11p (ref. 8). We have now studied the organization and copy number of sequences at three loci mapped to 11p: c-Ha-ras1, insulin and γ -globin in cells from four individuals with structural rearrangements of the short arm of chromosome 11. Our results reported here rule out a close physical linkage between c-Ha-ras1 and the genes responsible for AWTA, and suggest a more distal localization of the β -globin cluster than currently assumed.

WT is usually sporadic, but may be inherited as an autosomal dominant trait with reduced penetrance⁹. The gene has not been formally mapped, but its association with aniridia points to the short arm of chromosome 11 (ref. 7). Aniridia—absence or incomplete development of the ocular iris—is an extremely rare birth defect; and when associated with an 11p13 deletion it indicates a high risk (~30%) for developing WT⁸. Twenty-seven cases have been reported with aniridia and variable deletions of 11p, all involving absence of the distal half of band 11p13 (ref. 10). Twelve of these patients had WT and two had gonadoblastoma.

Although we have shown that c-Ha-ras1 is located far from the deletion responsible for predisposition to WT, it cannot be excluded that the rearrangement could alter the expression of this oncogene or of other oncogenes.

We thank Professor J. C. Kaplan for helpful discussion, Drs J. de Grouchy, C. Turleau, C. Laurent, J. Fraisse, T. Philip and J. Boué for help in obtaining the tissues used in this study, and Drs E. Chang, F. Ramirez, J. Myers and A. Bank for providing cloned DNA fragments.

Note added in proof: At the Los Angeles VIIth International Workshop on Human Gene Mapping (21–26 August, 1983), the gene for *HBBC* was shown to map to 11p15, and not to 11p12. The gene order is therefore: centromere–*LDHA*–*CAT*, *AWTA*–*HBBC*–*INS*, c-Ha-ras1. Our results are in good agreement with this gene order.

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Our previous localization of c-Ha-ras1 spans almost the entire short arm of chromosome 11 and includes band 11p13 which plays a critical part in predisposition to WT. To determine whether the oncogene could be directly involved in tumorigenesis we have now analysed fibroblasts of a patient previously described¹¹ as having aniridia, multiple anomalies, mental retardation and 11p deletion and lymphoblasts of her mother who has a balanced shift of 11p material to the long arm of the same chromosome (GM 5912), her half-brother who has triplication of the same material (GM 5913)¹², and an unrelated female with AWTA and 11p deletion (GM 5518)¹³. The three lymphoblast lines were obtained from the Human Genetic Mutant Cell Repository at the Institute of Medical Research, Camden, New Jersey.

When chromosomes were re-examined in our laboratory at the time of DNA analysis, the pertinent abnormalities were confirmed and their interpretation refined (Fig. 1). In GM 5912, region 11p11.2→p14.1, comprising about one-third of the short arm of chromosome 11, has been inserted into 11q at band 11q22.2 (Fig. 1a, e). This balanced shift of chromosomal material is associated with a normal phenotype. In meiosis, an uneven number of cross-overs between bands 11p11.2 and 11q22.2 has led to two different types of unbalanced offspring. Individual B with aniridia has inherited a chromosome 11 with a shortened short arm but a normal long arm (Fig. 1b), whereas individual C has received a chromosome 11 with a normal short arm and an elongated long arm due to insertion of the duplicated short arm material (Fig. 1c). Both children received a normal chromosome 11 from their respective fathers. Region 11p11.2→p14.1, which contains the genes predisposing for aniridia and WT, is thus represented in this family in one copy, two copies and three copies respectively. The cytogenetic interpretation is supported by biochemical gene dosage studies^{11,13}. Individual B has partial lactate dehydrogenase A deficiency (*LDHA* locus on 11p12)^{11,14} and individual C has increased catalase activity (*CAT* locus on 11p13)^{13,15}. The unrelated individual GM 5518 has AWTA and a complex rearrangement between the short arms of chromosomes 9 and 11 resulting in a net deletion of region 11p12→p14 (Fig. 1d), as indicated by half-normal catalase levels¹³.

DNA samples prepared from total cellular DNA were cleaved with different restriction enzymes, and analysed with probe pEC,

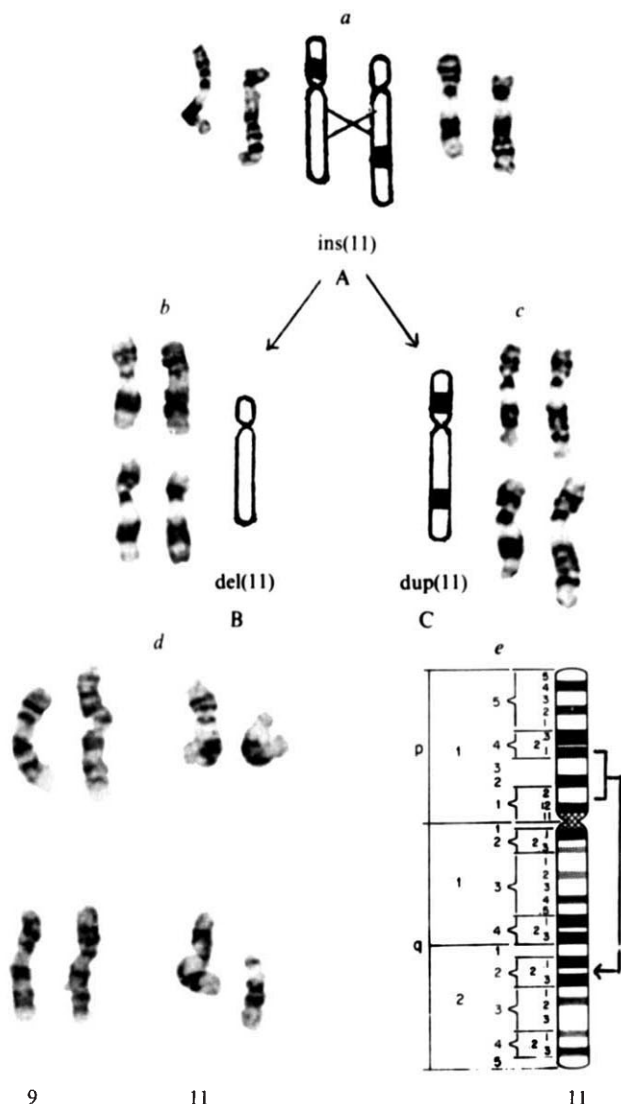


Fig. 1 Trypsin-Giemsa-banded partial karyotypes of the human cell lines used, and diagrams of chromosomes 11 indicating meiotic cross-over events in individual A which gave rise to deletion in individual B and duplication in individual C. *a*, Chromosome pairs 11 of GM 5912 with balanced intrachromosomal shift, designated inv ins¹¹ (p14p11.2;q22.2); *b*, chromosome pairs 11 of individual with aniridia and deletion of the material involved in the shift; *c*, chromosome pairs 11 of GM 5913 with duplication of the shifted material; *d*, chromosome pairs 9 and 11 of cell line GM 5518 with a complex t(9;11) translocation resulting in a net deletion of region 11p12→p14; *e*, idiogram of banding patterns on human chromosome 11 (ref. 28), demonstrating breakpoints of the insertion in A. In each chromosome pair the normal homologue is placed on the left.

a plasmid containing the normal cellular homologue of the transforming sequence of the EJ bladder carcinoma cell line¹⁶. With this probe, a restriction-fragment length polymorphism can be detected in *Bam*HI-, *Bgl*II- and *Taq*I-cleaved DNA samples, but not in *Eco*RI-cleaved samples. The most common allele is a fragment of 6.6 kilobase pairs (kbp) in *Bam*HI, ~8 kbp in *Bgl*II and 3 kbp in *Taq*I digests¹⁷. The next most common allele is of larger size and apparently results from DNA insertions of variable lengths^{17,18}. In heterozygotes for these two alleles the size difference between the fragments is constant in different endonuclease digests. The larger fragment may contain additional *Taq*I sites, and therefore can be further resolved by cleavage with this endonuclease. Autoradiograms (Fig. 2*a-c*) reveal that all DNA samples, including the EJ and T24 cell lines, but with the exception of one control, carry the common allele. In the insertion 11 family (lanes 2-4), the second fragment

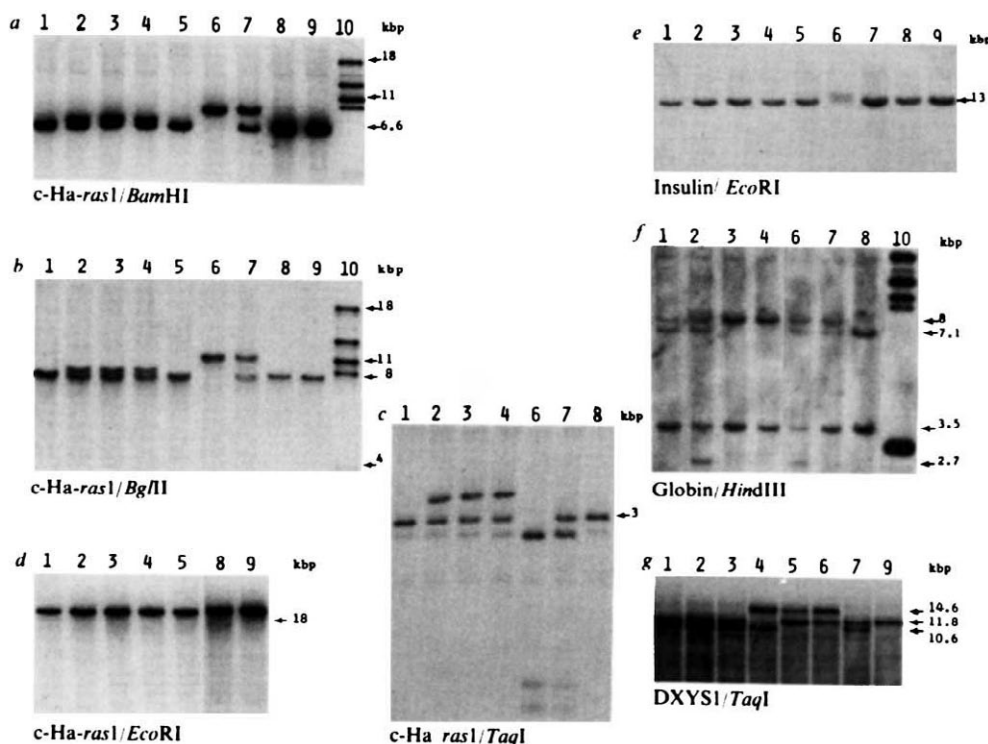
is ~1 kbp larger and does not contain extra *Taq*I sites. The fragments are of comparable intensities in the three family members in each autoradiogram. The unrelated individual with 11p deletion also had two fragments of equal intensity (lanes 7), the common allele and a larger one which is further resolved by *Taq*I cleavage. One normal male control was homozygous for this allele (lanes 6). Thus, two unrelated individuals who are hemizygous for 11p13 and for adjacent chromosome bands (11p12 and/or 11p14.1) have two restriction fragments which represent two alleles. The third individual with duplication of the same region also has two alleles. Furthermore, the single band in the *Eco*RI-cleaved DNA samples of the insertion 11 family (Fig. 2*d*, lanes 2-4) was of equal intensity rather than showing a 1:2:3 ratio of intensities, which argues against a gene dosage difference in this family.

The c-Ha-ras1 locus, confirmed to be on chromosome 11 by two other groups of investigators^{19,20}, must be located outside of the regions deleted or duplicated in these patients. It could either reside near the centromere in band 11p11.12, or more likely, in the distal region 11p14.2→pter. As a single chromosome band may contain 2,000-3,000 kbp, our data rule out a close physical relationship between c-Ha-ras1 and the sequences responsible for WT predisposition. Thus either c-Ha-ras sequences do not play a part in the development of WT, or if they do, activation of the protooncogene is not mediated by physical removal of one allele. It is well established that sequences located in band 11p13 predispose to WT specifically when present in the hemizygous state. The situation may be analogous to that described for retinoblastoma, where the respective sequences reside on chromosome 13 in band q14 (ref. 10) and deletion of this band predisposes to the development of retinoblastoma. No active transforming sequences have so far been isolated from either WT or retinoblastoma. These embryonic tumours may arise by another mechanism besides activation of cellular protooncogenes¹⁰. At any rate, an involvement of the c-Ha-ras1 oncogene in the development of WT is not suggested by its chromosomal location. Localization of both genes on the short arm of chromosome 11 may well be fortuitous.

Southern blot analyses were carried out with two further chromosome 11 short arm probes. A probe containing the human insulin gene²¹ revealed no restriction-fragment length polymorphism in most samples using several restriction enzymes. We observed a single band of equal intensity in both euploid and aneuploid individuals (Fig. 2*e*). The lack of demonstrable gene dosage makes it unlikely that the insulin locus is in region 11p22.2→p14.1, and is consistent with its reported²² *in situ* localization to band 11p15.

The β -globin cluster was studied with a γ -globin probe²³. Individual B with the inherited 11p deletion carries two different alleles (Fig. 2*f*, lane 2). One is inherited from the mother (8- and 3.5-kbp *Hind*III fragments; Fig. 2*f*, lane 3). The paternal allele (7.1- and 2.7-kbp fragments) represents a common polymorphism³¹ also present in one control sample (Fig. 2*f*, lane 6). The unrelated individual with 11p deletion also has two different γ -globin alleles (Fig. 2*f*, lane 7), one of which consists of a single 7.1-kbp *Hind*III fragment. These data indicate that the γ -globin genes have not been deleted in the two unrelated patients who are missing region 11p11.2→p14.1. We have previously reported another patient with interstitial deletion 11p11.1→p1203 who was heterozygous for haemoglobin S¹⁴. All our data taken together exclude the β -globin cluster from region 11p11.1→p14.1. Our results are inconsistent with the reported assignment of the β -globin cluster to region p11→p1208 that is based on studies of X-irradiation-induced deletions of 11p in Chinese hamster-human somatic-cell hybrids²⁴⁻²⁶ and on *in situ* hybridization³². Consistent with conclusions from studies of sorted translocated chromosomes²⁷, we propose that the β -globin region is located in the distal third of the chromosome 11 short arm together with the insulin gene and c-Ha-ras1. Family linkage studies, making use of the high-frequency DNA polymorphisms associated with these three loci, may soon establish recombination frequencies and linear gene order. Correct

Fig. 2 Autoradiograms of nitrocellulose filters containing human DNA samples prepared as described previously³⁰, and cleaved with *Bam*HI (a), *Bgl*II (b), *Taq*I (c, g), *Eco*RI (d, e) and *Hind*III (f); each lane contains 8 µg of DNA, except on filters b, c, g (5 µg per lane). The filters were hybridized with plasmids pEC (ref. 16; a–d), pIns96 (ref. 21; e), pJW151 (ref. 23; f) or pDP31 (ref. 29; g). All plasmids were ³²P-labelled by nick-translation. Origin of samples in the respective autoradiograms: lanes 1, human female lymphoblastoid cells; 2, skin fibroblasts from female individual with 11p deletion; 3, female lymphoblastoid cell line GM 5912; 4, male lymphoblastoid cell line GM 5913; 5, male human skin fibroblast control; 6, male human lymphoblastoid cell control; 7, female lymphoblastoid cell line GM 5518; 8, EJ bladder carcinoma cell line; 9, T24 bladder carcinoma cell line; 10, size marker. The DNA controls in lanes 1, 5 and 6 were extracted from cells with two normal chromosomes 11. The same *Taq*I-cleaved DNA samples (g) were hybridized with a probe that detects the Y-(14.6 kbp) and X-(11.8 and 10.6 kbp) specific fragments of locus *DXYS1* (ref. 29) to confirm the presence of the sex chromosomes in the samples.



physical localizations are essential for comparative studies of the relationships between genetic and physical maps of human chromosomes.

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Infectivity of the cloned geminivirus genome requires sequences from both DNAs

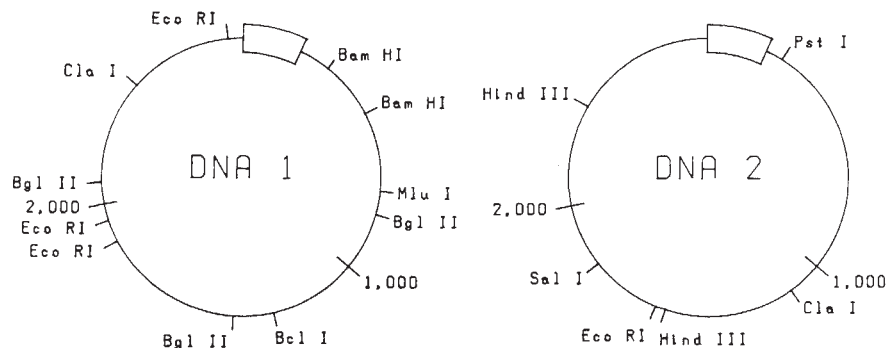
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Members of the geminivirus group are characterized by their twinned virions which encapsidate a genome of circular, single-stranded DNA¹. It has been suggested that the genomes of some members, specifically bean golden mosaic virus² and tomato golden mosaic virus^{3,4} are bipartite. Recently, the complete nucleotide sequences of two similar-sized, circular DNA molecules (DNAs 1 and 2) from cassava latent virus (CLV) have been determined in this laboratory⁵. There has been no unequivocal evidence, however, to show that two such DNA molecules represent the infective unit of the virus genome. Here I describe the construction of recombinant DNA clones that contain full-length copies of CLV DNAs 1 and 2 inserted into bacteriophage M13. The inserts, on excision from the cloning vectors, are together shown to be infectious in *Nicotiana benthamiana*, verifying the bipartite nature of the genome.

The two circular, single-stranded DNA molecules of CLV (West Kenyan isolate 844), DNAs 1 and 2, contain 2,779 and 2,724 nucleotides, respectively⁵. Although apparently separately encapsidated^{6,7}, the two DNAs are of sufficiently similar length and G+C content that they cannot easily be separated as either isolated DNA or while still encapsidated in the geminate particles. Therefore to investigate the contribution of each DNA to the process of virus replication, I decided to construct recombinant DNA clones containing full-length copies of DNAs 1 and 2 which could subsequently be excised in an intact form from the cloning vector. DNA polymerase-directed synthesis of a second DNA strand using the single-stranded virus DNA as a template has been described previously⁵. Restriction enzyme

Fig. 1 Restriction enzyme recognition sites in DNAs 1 and 2. Non-cutters include *Hind*III, *Pst*I and *Sal*I in DNA 1 and *Bam*HI, *Mlu*I, *Bgl*II and *Bcl*I in DNA 2. The open box represents the region common to both DNAs⁵.



sites within the double-stranded forms of DNAs 1 and 2 are shown in Fig. 1. Single-cutting restriction enzymes suitable for cloning full-length copies are *Mlu*I, *Bcl*I and *Cla*I for DNA 1, and *Pst*I, *Cla*I, *Eco*RI and *Sal*I for DNA 2.

Repeated attempts to clone DNA 1 in the *Bcl*I site of pKC7 (ref. 8) and in the *Cla*I site of pAT153 (ref. 9) were unsuccessful, although DNA 2 was cloned at its unique *Cla*I and *Pst*I sites in pAT153 in parallel experiments. In order to make use of the unique *Mlu*I site in DNA 1 it was necessary to develop a suitable cloning vector. M13mp8 (ref. 10) was chosen for this purpose, from which the vector M13mp8/*Mlu* was constructed as described in Fig. 2 legend. M13mp8/*Mlu*, in addition to the *Mlu*I cloning site, has retained the original *Pst*I and *Hinc*II sites of M13mp8. The insertion of the *Eco*RI molecular linker into the *Hinc*II site served both to replace the *Eco*RI site in the vector and to restore the correct reading frame of the *lac* gene, allowing screening of transformants by the *lac* complementation assay. Full-length copies of DNA 1 in both orientations (pJS091 and pJS092) were cloned at the *Mlu*I site in M13mp8/*Mlu*. DNA 2 was cloned in both orientations (pJS093 and pJS094) in M13mp9 (ref. 10) at the unique *Pst*I site. The reason for the inability to clone DNA 1 at the *Bcl*I and *Cla*I sites is unknown, but one possible explanation is that the intact virus DNA is expressed when in either orientation within the vector, and the products are detrimental to host bacterial multiplication, so that recombinants containing virus DNA inserts are produced but not detected. The recognition of eukaryotic promoters in such a prokaryotic system has indeed been envisaged in analogous cloning experiments concerning caulimoviruses¹¹. This argument requires that the products of expression of DNA 1, when cloned at the *Mlu*I site, are suitably altered (or eliminated) to allow *Escherichia coli* multiplication and hence detection of virus plaque formation. It may be significant that the *Mlu*I site is positioned within the region of DNA 1 thought to code for the coat protein⁵.

Linearized, double-stranded copies of DNAs 1 and 2, prepared as outlined in Fig. 3 legend, were mechanically transmitted to *N. benthamiana* either individually or as a mixture. The characteristic leaf-curling symptoms of CLV infection, shown in Fig. 3, were observed only when both DNAs 1 and 2 were present in the inoculum, all such inoculated plants showing symptoms. Thin sections of these systemically infected leaves revealed the presence of fibrillar ring structures within the nuclei of infected cells, a cytopathological effect typical of CLV and other geminiviruses^{12,13}. Geminate virus particles could be isolated from plants infected with cloned DNAs 1 and 2 and were infectious when reappplied to *N. benthamiana*. In addition, coat protein extracted from virus particles purified from plants infected with either a mixture of cloned DNAs 1 and 2 or virus DNA co-migrated on SDS-containing polyacrylamide gels and showed an identical immunoprecipitation response in double-diffusion plates towards antiserum raised against virus (data not shown).

To investigate the relative extent of DNA replication within inoculated plants, nucleic acids were extracted directly from systemically infected leaves (or their equivalent when symptoms were not evident), applied to nitrocellulose and hybridized to probes specific to each DNA (that is, cloned virus DNA exclud-

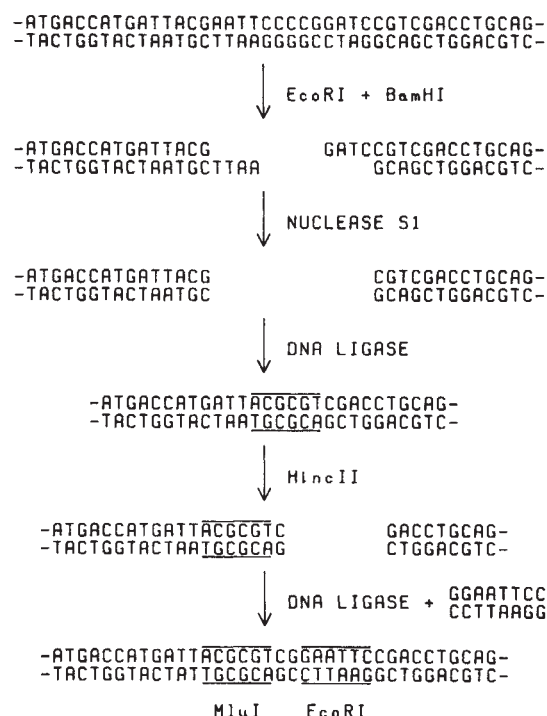


Fig. 2 Construction of cloning vector M13mp8/*Mlu*. The replicative form of M13mp8 (ref. 10) was completely digested with *Eco*RI and *Bam*HI, phenol-extracted and the DNA precipitated with ethanol. The double-digested DNA was treated with *S*₁ nuclease (2 units per μ g DNA) using the conditions described elsewhere¹⁴, phenol-extracted and ethanol precipitated. The vector was blunt-end ligated to create the *Mlu*I site and the DNA used to transform competent *E. coli* JM101. Colourless plaques were selected and screened for the presence of an *Mlu*I site after small-scale preparation of M13 replicative form¹⁵. Sequences of M13 DNA were verified using the dideoxy termination procedure¹⁶. A suitable recombinant was selected, the replicative form completely digested with *Hinc*II, and phosphorylated *Eco*RI molecular linkers were blunt-end ligated into the cut vector. After transforming competent *E. coli* JM101, plaques with restored blue colouration were selected and the M13 DNA screened for the desired sequence using the dideoxy termination procedure.

ing any of the region common to DNAs 1 and 2). As shown in Fig. 4a, only when both DNAs 1 and 2 were present could CLV DNA be detected above the nonspecific level of binding of the probe to DNA from the control, mock-inoculated plants. To analyse the form of the DNA, samples were extracted from purified geminate particles and run on an agarose gel both with and without previous treatment with dimethyl sulphoxide (DMSO) and glyoxal to denature the nucleic acid (Fig. 4b). Most of the encapsidated nucleic acid specific to CLV produced in plants inoculated with a mixture of cloned DNAs 1 and 2 (lanes 1, 3, 7 and 9 of Fig. 4b) co-migrates with native virus DNA from virus DNA-infected plants (lanes 2, 4, 8 and 10), showing that circular, single-stranded DNA can be synthesized from the initial inoculum of linear, double-stranded DNA.



Fig. 3 Systemic infection of *N. benthamiana* with CLV. The replicative forms of M13 clones pJS092 and pJS094 were prepared, the inserts excised using the appropriate restriction enzyme and purified by sucrose gradient centrifugation. *N. benthamiana* plants were maintained in a glasshouse at 25 °C. Two weeks after germination, 20 plants were either mock-inoculated or inoculated with 1 µg of cloned DNA (either DNA 1 and 2 separately or as a mixture) or with 1 µg of virus DNA (West Kenyan isolate 844) and systemically infected leaf tissue (or its equivalent in the case of non-infected material) was collected ~2 weeks later. Leaf tissue was stored at -70 °C until required for virus purification as described¹⁷. The plants shown were inoculated either with cloned DNAs 1 and 2 (left) or with virus DNA (centre), or mock-inoculated (right).

Furthermore, hybridization with probes specific to each DNA shows that both DNAs 1 and 2 are encapsidated in similar amounts. Presumably, on infection with the excised cloned DNA, the latter is ligated to give circular, double-stranded DNA, a putative replicative form of the virus DNA produced during the natural infection cycle of the virus³.

In addition to DNAs 1 and 2, several minor bands (arrowed) are present in Fig. 4b. As this material routinely appears in DNA extracts from geminate particle preparations and is also represented in the same relative amounts in total nucleic acid extracts of systemically infected leaves, it is not a result of contamination during virus preparation but would seem to be strongly associated with, or encapsidated in, the geminate particles. Data concerning susceptibility to nuclease digestion show that all of the nucleic acid within the minor bands is single-stranded DNA. The slower migrating material which is present in all samples is split into two bands. The material within these bands has not yet been fully characterized but it may represent either concatemeric forms of the single-stranded DNA, or unit-length single-stranded DNA tightly associated with coat protein. A minor band containing sequence in common with DNA 2 and approximately half its size is present in Fig. 4b (track 12). As this band routinely appears when plants are inoculated with virus DNA but not when inoculated with cloned DNAs, it possibly represents a non-essential, defective DNA 2 molecule that is replicated with the help of DNAs 1 and 2 and is propagated in the virus population by virtue of the fact that it can be encapsidated. Due to the limited size of the probes used, an analogous molecular species derived from DNA 1 may also be present but remain undetected.

In order to produce infective progeny virus, it has been demonstrated that both parts of the CLV genome, DNAs 1 and 2, must be present. This does not, however, rule out the possibility of independent replication of one or both of the individual DNAs, which would remain undetected when not transported systemically throughout the plant. To investigate the potential of each part of the genome for self-replication, experiments using mesophyll protoplasts are now in progress. As previous sequencing studies⁵ have shown that the original virus isolate used was composed of a heterogeneous DNA population, the construction of infective, full-length recombinant DNA copies of the CLV genome will allow the derivation of unambiguous

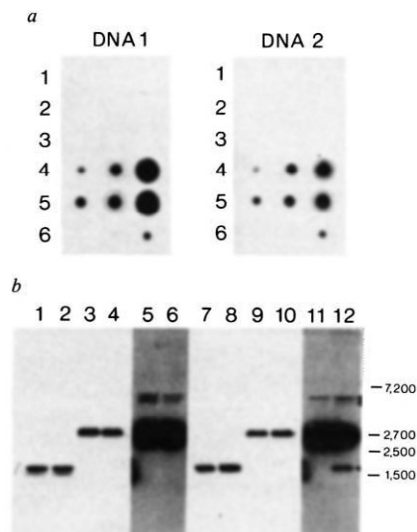


Fig. 4 The production of CLV DNA in *N. benthamiana*. Plants were propagated and inoculated as described in Fig. 3 legend. **a**, Nucleic acids were extracted from 1 g of frozen leaf tissue by homogenizing with 2 vol. of 100 mM Tris-HCl pH 7.0, 100 mM NaCl, 10 mM EDTA, 1% SDS, extracting the homogenate three times with one volume of phenol/CHCl₃ (8:2, v/v) and precipitating with ethanol. The precipitated material was redissolved in 1 ml of 50 mM Na-acetate pH 5.0. Samples of the extracts (1, 3 and 9 µl, made up to 9 µl with 50 mM Na-acetate pH 5.0) were applied directly to nitrocellulose and hybridized with nick-translated probes specific to CLV DNAs 1 (left) and 2 (right). The probes used were a *Bgl*III fragment (nucleotides 804–1,410) of DNA 1 and a *Hind*III fragment (nucleotides 1,494–2,281) of DNA 2 cloned in the *Bam*HI and *Hind*III sites respectively of M13mp9 (ref. 10). Plants were either mock-inoculated (1), or inoculated with DNA 1 (2), DNA 2 (3), DNAs 1 and 2 (4) or virus DNA (5). To quantitate the extraction, loadings of 1, 5 and 25 ng of single-stranded CLV DNA were made (6). **b**, Electrophoresis of nucleic acids on a horizontal 1.4% agarose gel. (After electrophoresis, the DNA was blotted onto nitrocellulose and hybridized with nick-translated probes specific to DNAs 1 (lanes 1–6) and 2 (lanes 7–12). The nucleic acid was extracted from geminate particles purified from equal weights of leaf tissue derived from plants inoculated with either cloned DNAs 1 and 2 (lanes 1, 3, 7 and 9) or virus DNA (lanes 2, 4, 8 and 10). DNA samples were either left untreated (lanes 1, 2, 7 and 8), or were treated with glyoxal¹⁸ (lanes 3, 4, 9 and 10) before application to the gel. Lanes 5, 6, 11 and 12 represent longer exposure times of tracks 3, 4, 9 and 10, respectively. In non-denaturing conditions (lanes 1, 2, 7 and 8), the DNA is resolved into its circular and linear forms. The size markers (approximate numbers of nucleotides) were derived from glyoxal-treated restriction fragments from the replicative form of the M13 clone pJS094.

sequences for DNAs 1 and 2 to serve as a basis both for the conception of experiments and the interpretation of their results.

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Digoxin-like immunoreactivity of a circulating $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ inhibitor

WE wish to dispute a statement made by Hamlyn *et al.*¹ concerning the use of anti-digoxin antibodies to detect a circulating inhibitor of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$. They claim to be using a technique which is "essentially as described by Gruber *et al.*". In reality the procedure they used is not our technique. We use plasma (4–5 ml) isolated from 10 ml of blood. The plasma is deproteinized, Diafiltered through Um-10 membranes, then the filtrate is lyophilized and redissolved in 500 μl of water². Our approach purifies the extract as we concentrate it. In the method described by Hamlyn *et al.* it is unclear whether they concentrate or dilute the deproteinized extracts. In addition, they do not mention a Diafiltration procedure.

In our experience, concentrating a deproteinized plasma extract without first Diafiltering it produces a sample with little immunoreactivity. In addition, the digoxin-like activity present is very variable, will not dilute out, and thus does not give parallel displacement to digoxin in serial dilutions.

Parallelism is the cornerstone of any radioimmunoassay (RIA), especially when attempting to measure a cross-reacting factor. In our original publication², we demonstrated that as plasma extracts were purified, their serial dilution displacement curve approached that of standard digoxin. A Diafiltrate was the crudest extract with which we could obtain an acceptable displacement curve.

Hamlyn *et al.* go to great lengths to verify their $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ assay. It is a pity they did not go to similar lengths to verify that their RIA was detecting the same substance that we first reported on. As they provided no evidence or inference that they were able to dilute out their endogenous digoxin immunoreactivity, it is conceivable that their measurements were made on the nonlinear part of the displacement curve; it is impossible to quantify immunoreactivity unless it is read on the linear segment of the dilution curve. Other investigators have approached the detection of an endogenous digoxin-like factor in the appropriate manner³, and have confirmed our original findings.

In view of the comments made by Hamlyn *et al.*¹ concerning the results of their digoxin RIA, we feel it is important to draw attention to the fact that the excellent correlation they show between $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ inhibition and blood pressure in normotensive and hypertensive humans is strikingly similar to that which we first obtained by measuring digoxin-like immunoreactivity in monkeys with spontaneous hypertension⁴. Elevated digoxin-like activity has also been found in plasma of rats with coarctation hyper-

tension⁵. Finally, injection of anti-digoxin antibodies lowers the blood pressure of deoxycorticosterone acetate-salt hypertensive rats⁶.

The measurement of a cross-reacting substance by a radioimmunoassay is at best a difficult procedure. To not follow an established technique is to invite failure unless one wishes to establish the validity of one's own assay procedure.

Note added in proof: Hamlyn *et al.*⁷ have recently reported that endogenous digoxin (endoxin) levels directly correlate with plasma noradrenaline and inversely correlate with aldosterone. Thus, endoxin appears to have some physiological variability.

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HAMLYN *ET AL.* REPLY—Gruber and Buckalew are correct in asserting that the method we recently used¹ to measure digoxin-like immunoreactivity (DLI) in samples derived from the plasma of normotensive and hypertensive individuals, is not identical to the one they described². The differences between the two procedures, however, are smaller than claimed by Gruber and Buckalew and hence led us to use the phrase "essentially as described"¹.

The methods we used to prepare the plasma samples for the digoxin radioimmunoassay (RIA) are clearly stated in the legends of Figs 2 and 3 of ref. 1 and differ from the Gruber *et al.* procedure² only in that we omitted the ultrafiltration step used by them. This omission was based on an earlier report by Buckalew³ which showed that an inhibitor of active sodium transport obtained from dog plasma can bind to the polypropylene membrane support used in the ultrafiltration apparatus. It seemed unwise to use such a protocol without the benefit of a stainless steel support³ because the recoverability of active material is then difficult to determine. Furthermore, the claim that Diafiltration removes interfering substances appears to compound the difficulty in quantitating the immunoreactivity of native plasma. It is noteworthy that we did not experience any difficulty in detecting, quantitatively, the small amounts of digoxin (50–100 pg ml^{-1}) that were added

to either native or deproteinized plasma samples, as 'internal standards' for the digoxin RIA. Thus, any interfering substances must inhibit only the interaction of the digoxin-like material, and not digoxin itself, with the antibody.

The claim that other investigators have used appropriate methods to quantitate DLI is not directly relevant. The source of material (guinea pig heart versus plasma from volume-expanded dogs) and the extraction protocols used by others in the preparation of DLI⁴ were very different from that reported by Gruber *et al.*². Consequently, the work by DePover *et al.*⁴ cannot be considered direct confirmation of the original findings of Gruber *et al.*².

The specificity of the anti-digoxin antibody may also be a problem. This antibody shows little or no cross-reactivity with other cardiac glycosides. In contrast, the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ will bind, and is inhibited by, a wide variety of structurally different cardiac glycosides. This implies that the anti-digoxin antibody is not recognizing the structurally important determinants involved in inhibition of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$. Thus, the relationship of the digoxin-like material to inhibition of the ATPase by endogenous factors is not clear.

We found¹ that human plasma (when deproteinized and concentrated) contains a DLI substance. However, as reported, we found no significant difference in plasma DLI between normotensives and patients with essential hypertension. The immunoreactive factor did dilute out and, in contrast to the experience of Gruber and Buckalew, was not variable in repeated assays.

The significance of the relationships between blood pressure and DLI in spontaneously hypertensive monkeys⁵, and between $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ inhibition and blood pressure in human essential hypertension¹, alluded to by Gruber and Buckalew, are complicated by the use of different assays, and by the uncertainty that the primate (or rat) model is an appropriate model of the human disease. In our view, the appropriate way to quantitate endogenous inhibitors of the sodium pump is to use the pump molecule itself in the assay system.

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Australasian archaeology

J.V.S. Megaw

A Prehistory of Australia, New Guinea and Sahul.

By J. Peter White and James F. O'Connell.

Academic: 1982. Pp.286. £19.60, \$29.50.

Archaeology of the Dreamtime.

By Josephine Flood.

Collins: 1983. Pp.288. £13.95, \$24.95.

IN THE publication explosion of the last twenty years it might be assumed that archaeology would have been well-served by general introductions and overviews of this or that ancient people and this or that ancient place. The truth, as so often in publishing, is not quite so simple. For example there is no doubt that for world overviews and beginners' guides the United States has scooped the pool, with Grahame Clark's *World Prehistory* (Cambridge University Press), a notable British exception. However, it is impossible to obtain an up-to-date survey of the prehistory of Europe or, until comparatively recently, a comprehensive review of the British Isles. Is it that European archaeologists are less worldly than their American counterparts; or may be they have less undergraduate teaching to do? Is it that European archaeologists believe they know too much; or rather that after some two centuries of serious research in the field there is too much to know?

Certainly by these criteria Australasia may count itself fortunate. The first full-time tertiary appointments in archaeology and prehistory — any sort of archaeology, any sort of prehistory — date back a mere 25 years. In 1961, the year which saw publication of the first edition of Clark's *World Prehistory*, John Mulvaney, now doyen of Australian academic prehistorians, was writing of Australia as "the dark continent of prehistory". By 1975, the revised and much expanded edition of Mulvaney's *The Prehistory of Australia* (Thames and Hudson) first published in Britain in 1969, had been issued by Penguin Books. This is now out of print and was inexplicably never generally available outside the Southern Hemisphere. Historians also added their view of Australia's distant past; Geoffrey Blainey's *Triumph of the Nomads: A History of Ancient Australia* (Macmillan) was also published in 1975.

The first of several briefer accounts appeared in the same year, J. Peter White's *Before the White Man: Aboriginal life in Prehistoric Australia* published in Sydney by Readers Digest — not referred to by

White in his new survey, though cited by Josephine Flood. Two years later Clark devoted some 50 pages of the latest edition of his world survey to the prehistory of Australasia and Oceania, some the product of research by his own pupils of whom John Mulvaney is one, others including two of the three authors of the books here under review. (Some, but not all the pioneers of modern prehistory in Australia are Cambridge graduates, but how far Cambridge in the bush, economic



A double-stranded band of notched wallaby teeth encircled the forehead of this man buried at Roonka on the River Murray, South Australia, over 4,000 years ago. (From *Archaeology of the Dreamtime*.)

prehistory sometimes labelled 'ecological determinism', has really controlled the destiny of modern Australasian archaeological research can be debated.)

The archaeology of Australasia is now certainly newsworthy. Rhys Jones' film for television, *The Last Tasmanian*, produced and directed by Tom Hayden gained world-wide distribution and continues to engender local notoriety; the same one-time wild colonial boy has also provided the material for two articles on the First Australians in the Australian edition of *Playboy Magazine* — coyly hidden in White and O'Connell under 'Deiley, R. 1979'. More recently the Gordon-below-Franklin hydro-electric scheme has involved archaeologists in confrontation politics and front-page stories.

Where then is one to place these two new surveys of the prehistory of the Great South Land? Their titles and format in fact give a very fair indication of their different appeal and presumably the rather different

markets for which they are aiming despite covering very similar material. Both books are produced by the Sydney offices of international publishers, White and O'Connell with the economical double-column setting and generally drab but serviceable — though in the case of the comparatively few half-tone illustrations, distinctly grey — house style of Academic Press, a firm which seems erroneously to assume that scholars and students alike are uninterested in good design and attractive lay-out. White and O'Connell's chapters have brief, indeed terse, descriptions whereas Flood prefers more journalistic titles. Neither volume, despite much citing of radio-carbon dates, is particularly easy to follow chronologically. Flood lacks the numerous line drawings of artefacts included in White and O'Connell, well-executed by Margrit Koettig, but compensates by a large number of excellent colour photographs.

The front cover of *Archaeology of the Dreamtime* shows an Aboriginal informant against the spectacular backdrop of the Arnhem Land Escarpment, one of the major sources for the great wealth and variety of prehistoric Australian parietal art. Living Aborigines are virtually absent from White and O'Connell and they play down art largely on the presumed grounds that 'its correlation with other aspects of prehistory is still only beginning to be explored'. While they do acknowledge just such recent work in North Queensland with artefactual evidence associated with engravings of at least 13,000 BP, Flood's account is much fuller as one should expect of one who has herself recently carried out research in the same region for

the Australian Heritage Commission. This fuller coverage and her almost chatty style, adopted in the desire to write "for the general reader, for Aborigines interested in learning more of their own heritage, and for secondary and tertiary students" suggests that here indeed will be the overview with the potential to reach a wider audience.

But content is supposed to be more important than packaging. For those interested in the history of Australian prehistory, one should expect from White, like his teacher Mulvaney (both first trained as historians at Melbourne), a useful overview. Mulvaney has done the job better, however; his paper 'Prehistory from Antipodean Perspectives' contributed to a collection for Grahame Clark (*Proc. Prehist. Soc.* 29, 2: 1971) which might have been noted and so too his 'Australia before the Europeans' (*Bull. Inst. Archaeol. Univ. London* 15: 1978) in which Vere Gordon Childe, Australia's greatest archae

ologist, is assessed from an Australian view-point. Indeed, though Childe receives not a single mention in either book, and although in his life-time he ignored Australia almost as much as it did him, during his fatal return home in 1957 he recognized the need for an expansion of endeavour in Australian archaeology. At the present his contributions to social theory are commending themselves to the current generation of neo-Marxist anthropologists and archaeologists in the Southern Hemisphere — for example, N. Thomas, 'Social Theory, Ecology and Epistemology' (*Mankind* 13, 2: 1981).

Although White and O'Connell make considerable play of perceived theoretical weaknesses in the work of others and the need for looking at data in terms of general theoretical constructs, for two early champions of 'Australasian' ethnoarchaeology they are remarkable inconsistent in their regard for the ethnographic record, for example when examining human-environment relationships. No Australian, certainly no Australian archaeologist, is free from the grand Antipodean sport of rubbishing one's mates; perhaps with White and O'Connell it is more an attitude of 'holier than thou' which occasionally obtrudes. But there is enough referenced material which is quoted in both volumes to allow those who wish to proceed to piece together the past from other sources — though citation of a great deal of otherwise unpublished material, including undergraduate and graduate theses as well as unpublished conference papers to which White and O'Connell have had access, will surely frustrate many. For up to the minute reporting Flood undoubtedly has the edge; she presents, for example, some of the recent evidence for Pleistocene occupation in inland south-west Tasmania (*Nature* 301; 6 Jan. 1983).

White and O'Connell offer compensation — if it can be so regarded — in their somewhat curious *Appendix I* 'Uses of the past: Tasmania', quoting correspondence which arose in reply to a remarkably ill-informed and ill-conceived piece on the Tasmanian Aboriginal — not written by an archaeologist or anthropologist — which appeared in 1982 in the pages of the Sydney weekly journal *The Bulletin*, a fact which the authors curiously see fit not to reveal. If their purpose was indeed to show how theories of the past can affect the present then a much more significant public debate based on archaeological interpretations was that which followed, some five years ago, the first screenings of *The Last Tasmanian*.

Both volumes deal extensively with the continuing major themes of Australasian prehistory such as the still inconclusive debate on the first human settlement of what White and O'Connell refer to as 'Sunda'. (The conjoined land mass of New Guinea, the Australian mainland and Tasmania formed one unit 25–12,000 BP as marked today by the submerged Sunda

Shelf.) Flood, citing the recent Willandra Lakes robust hominid WHL 50 possibly of at least 30–25,000 BP, follows the gracile-robust dual theory of Alan Thorne who relates the two types to successive streams of ultimately mainland Asian *Homo erectus* origin. White and O'Connell argue against this but in my opinion, are no more convincing.

On the long-distance morphological links in the early 'core-and-flake' stone industries of Australasia, Flood is able to describe and illustrate not only the so-called 'Kartan' industry of Kangaroo Island, in the Pleistocene part of the southern land-mass, but the implements recently discovered by Les Groube on the raised coral terraces of the Huon Peninsula of Papua New Guinea. The terraces are dated to 50–40,000 BP and if this is also the date of the waisted axes found by Groube, it provides even more striking evidence for the Pleistocene occupation of the region than the similar forms excavated by White at Kosipe in the south-east New Guinea Highlands dated to 26,000 BP. One awaits eagerly further evidence from the Huon Peninsula because this equals the earliest date generally accepted from the Australian mainland for human settlement, that from the Upper Swan Bridge in south-west Western Australia.

White and O'Connell's main story on the prehistory of New Guinea and its integration with the rest of 'Sunda Land' is one of the best parts of their account. I am less certain that every reader will want to follow their more extended debate on the human history of Tasmania. Here they use the ethnographic analogy of Highland New Guinea as an area where a simple technology is associated with a complex culture to explain the restricted artefact range of post-Pleistocene Tasmania — in contrast with that of the mainland — as well as the sudden disappearance of fish from native diet.

On the question of megafaunal extinctions, Flood again is able to cite the most recent evidence, in this case from Tambar Springs in western New South Wales. Artefacts, including in later levels 'Kartan' material, are associated with megafauna which is a strong indication of hunting activities. Nonetheless Australian evidence certainly seems to argue against any simplistic and generalized 'overkill' theory for the disappearance of certain fauna, not least because, as White and O'Connell make clear, it seems that within each genus it is only the larger species which have died out.

The books are not all artefacts and ancient economics; the finer things of prehistoric life are not totally forgotten. Flood has two chapters on art and technology, one for the pre-Holocene period and the other for the last thousand years. White and O'Connell seem less at ease in such areas, though tucked away with 'other tools' in their pre-10,000 BP chapter they offer a succinct summary which empha-

sizes the early evidence, not only for varied burial practices but also self-decoration and forms of linear art of older than 20,000 BP. Equally, both volumes contrast the general similarity of the material culture of all areas in the Late Pleistocene with the variety which is found at later periods.

To account for these changes for this material and particularly for what Mulvaney has termed the 'small tool tradition' of c.5,000 BP, White and O'Connell offer an adaptation "primarily for stylistic and social reasons" — though what those reasons might be is again far from clear. Flood argues for the long established though certainly non-proven theory that the small tool tradition has a source in the Indian sub-continent or in mainland south-east Asia. White and O'Connell do offer a useful precis of probable events in this later period which relates regional variations in tool types and burial practices to the as yet largely undated variations in rock art styles. The sophistication of a community capable of utilizing as a food source the highly poisonous macrozamia nut and, in eastern Melanesia at least, a marked increase in the occupation of highland sites suggests not only improved aspects of food technology and trade in raw materials but external social factors such as regionally-organized ceremonies.

With these last topics one approaches the people as well as the artefacts but it cannot be said that either book has given us, or can give us, the Aboriginal past itself. It is, alas, true that we are a long way off the informed production of an Aboriginal-written perspective of prehistory. We are not yet completely free even of the long-lasting view chronicled elsewhere by White (*World Archaeology* 13, 2: 1981) that the Aboriginal is a mirror of the distant past. Fifteen years ago Rhys Jones, writing an editorial in *Mankind*, stated "The honeymoon is over. The new wave of Australian archaeology is settling down comfortably to a premature middle age". Now that the Young Turks of the 1960s are all middle-aged I do not myself detect any premature fossilization in either of these surveys of Australasia's complex past.

When, many years ago, I was working as an editor on one of the first of a series of archaeological coffee-table books I was admonished by a successful American publisher who, glancing at my caption copy, demanded 'More excitement, more blood, more sex'. Neither Flood nor White and O'Connell exhibit much sex let alone sexism, neither manages to squeeze much blood out of their mute stones but it is in my view Flood who does manage to impart something of the excitement of working in an area which still can be called *Terra australis incognita*. □

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Rocking the boat

Robert Ubell

Naked Emperors: Essays of a Taboo-Stalker.

By Garrett Hardin.

William Kaufmann/W.H. Freeman:
1983. Pp.280. Hbk \$15, £12.95;
pbk \$8.95, £6.95.

THIS is a work of great mischief. Wrapped in the cloak of science, it cannot totally conceal its naked racism and chauvinism. If Garrett Hardin did not have a large following among ecologists and population and resource management experts, one would be tempted to dismiss his vulgar exploitation of biology. But just as Herman Kahn's facile talk of nuclear war games made a holocaust appear palatable at tea-time, so Hardin's glib prescriptions for salvation permit us to accept starvation, sterilization and civilized savagery.

Hardin first came to prominence with the publication of his undergraduate biology textbook in the late 1940s. In it, he claimed that because people with the most education have low reproduction rates and those with low IQ scores breed profligately, we should expect a continuous downward trend in average intelligence. To avoid the consequences of this effect, he adopted the tenets of the eugenics movement and called for "measures aimed at discouraging the breeding of less-desired types of humans", mainly by sterilization. Hardin is much tougher than his Social Darwinist predecessor, Herbert Spencer, who merely said, "If the poor died of their own foolishness, the species would improve".

In the 1970s Hardin went on to promulgate his famous "lifeboat ethics". A seductive metaphor, it drew a picture of two lifeboats bobbing on the sea. In one were the rich nations, comfortably basking in wealth and culture. In the other, overcrowded, boat were the poor people of the world — continually falling into the water, flailing about, clamouring to be hoisted aboard the privileged vessel. According to Hardin, the wealthy nations are not merely fortunate: they are guardians of "civilization and dignity". So their task is to keep their boat afloat, preventing it from sinking under the weight of the poor. If the rich do not withhold resources from those who need 'help' to survive, the poor will inevitably over-breed, making a bad situation worse. We will all go under, unless we

"let 'em starve" as the *National Observer* once characterized Hardin's scheme.

Hardin offers a simple analysis: food and energy are scarce; overpopulation stretches the limits; the wealthy few must protect their preserve from the breeding poor. But he neglects to inform us that the United States alone, with only 5% of the world's population, consumes about 40% of the world's aluminium, nearly 30% of its iron, more than 60% of its natural gas, and a third of its petroleum. It seems rather perverse to condemn the poor for profligacy.

Moreover, it would seem that the underdeveloped nations can do without the 'help' they receive from industrialized parts of the world. In 1981, foreign investment in the Third World amounted to

\$62.6 billion; but profits repatriated to investor countries came to \$139.7 billion.

In *Naked Emperors*, a collection of recent articles written for professional journals and for such popular American magazines and newspapers as *The New Republic* and the *Los Angeles Times*, Hardin continues to cling to his early themes. Because he holds a professorship in human ecology at the University of California at Santa Barbara, is frequently seen on television, and speaks from the pulpit of scholarly periodicals, Hardin is taken seriously. It was Ortega y Gasset who once said, "Contemporary science. . . can put blockheads to good use". □

Robert Ubell, until recently the American publisher of Nature, is head of a science publishing house in New York.

Dialogue in motion

K. E. Machin

Animal Mechanics, 2nd Edn.

By R. McNeill Alexander.

Blackwell Scientific: 1983. Pp.301.
£15, \$25.95.

ALTHOUGH mechanics is undoubtedly part of physics, the relatively new and fashionable subject of biophysics has never laid claim to animal mechanics, which has therefore been able to lie low in zoology departments and in the pages of the *Journal of Experimental Biology*. Long may it remain so: animal mechanics flourishes best where the importance and interrelation of form and function are understood, with natural selection providing the ultimate incentive for getting the design right. Actually animal mechanics is not so much a subject as an approach — looking at the animal kingdom through a physicist's eyes. Even an approach needs a good textbook; this is it.

Modern biologists, we all agree, must be numerate; we are delighted when a biology undergraduate arrives armed with "A" levels in physics and mathematics. Yet all too often we fail to build on this good foundation; the undergraduate may even revert to the wild-type and develop a phobia for things quantitative. We need to find a better way to teach biologists to think quantitatively.

In the first edition of this book (published in 1968) Alexander did just this, inventing a method of feeding mechanics to zoologists in a very palatable form:

Each chapter consists of alternate sections on mechanics and zoology: a section explaining a group of mechanical principles is followed by one or more sections describing zoological investigations in which those principles have been used.

Had he been writing four hundred years ago, Alexander would no doubt have started his title with "Dialogue . . .". The

mechanics might have been put into the mouth of a middle-aged natural philosopher — a rather formal, didactic man; his companion — a young, quick-witted naturalist — avidly seizes each new chunk of physics and applies it with enthusiasm and delight to his zoological observations. Nowadays, I suppose, the physicist and zoologist would be carried on the left and right channels of a stereo radio programme. But whatever the medium, the pedagogic technique works: before the zoologist can get too bored with equations, he is whisked back into his own territory to see the physics actually working on animals. One learns a subject by using it. In this way, Alexander introduces statics and dynamics, mechanics of machines, elasticity and strength of materials, viscosity and surface tension, hydrostatics and fluid dynamics, vibrations and sound. These topics, potentially dull as an engineering course, come alive through the zoological examples.

Those who have for years been using the first edition of *Animal Mechanics* will ask: how different is this new edition? The approach, the chapter headings and much of the material will be familiar. But the subject has not stood still: several new topics have joined the "mechanics" sections, and many new zoological examples have been introduced. There are many worthwhile changes of detail: the layout, typography and physical make-up of the book have improved notably; SI units are used throughout (editors of biological journals, please copy); the occasional infelicity has been tidied up; the index is vastly improved.

In his preface, Alexander says: "I was sorry to realize when I had completed this edition, that little mention of the work of Sir James Gray remains in it". He need not worry: the whole book is imbued with Gray's spirit. □

K.E. Machin is a Lecturer in the Department of Zoology, University of Cambridge, and a Fellow of Queens' College.

● The second edition of *Geothermal Energy* by H. Christopher H. Armstead, published by E & F.N. Spon, is now available. The first edition was reviewed in *Nature* 284, 380; 1980.

Erratic swimming

John J. Videler

Fish Locomotion.

By R. W. Blake.

Cambridge University Press: 1983.

Pp.208. £29.50, \$57.50.

THE text on the jacket of *Fish Locomotion* tells of discussion of a wide variety of subjects to be found inside, from basic concepts to new developments in this area of research. The book is intended to survey and review some of the more important aspects in the field, and so to be of interest to students of fish biomechanics, functional morphology, behaviour and ecology. The detailed list of contents is promising, too, covering basic fluid dynamics, muscle physiology and morphology, kinematics and energetics of different modes of locomotion, as well as 'hot' topics such as optimal swimming strategies.

Alas, the book itself is very disappointing. The preface starts with the nebulous statement that Aristotle anticipated Newton by twenty centuries in recognizing the physical basis of the process of undulatory swimming. Such a statement irritates when there is no attempt to support it and this irritation does not vanish as one delves further into the book. I will direct my criticism to three aspects.

First there is the explanation of important concepts, several passages giving the impression that the author has an incomplete grasp of the theories he is attempting to elucidate. In the account of induced drag, for example, faulty trigonometric deduction is clearly caused by a slovenly drawing but also reveals a superficial understanding of the phenomenon. The explanation of Weihs' theory of the energetic advantages of burst and coast swimming is partly wrong and again shows a lack of understanding of the original paper.

The second fault is that, throughout the book, the literature is misquoted. For example, Rosen and Cornford (*Nature* 234, 49; 1971) presented data on the ability of fish slimes to reduce friction of the water. They included one measurement of the nut brown Cowrie: a mollusc. Not only does Blake consistently refer to the authors as Rosen and Cornfield, but he describes the cowrie as "a very slow swimming fish". The time interval between the images of swimming plaice larvae (Figs 131, 132) based on a paper by Batty (1981) is not 0.028 but 0.014 s.

Finally there are the figures and graphs, many of which show signs of carelessness. In 1933 Gray published a series of elegant and accurate drawings of cine pictures of swimming fish, and used them again in his 1968 book on animal locomotion. There are many more filmed records now available but Blake uses these pictures,

modified in such an ugly way that it is hard to recognize the original drawings.

This book seems to have been written and produced under pressure, every aspect of it betraying haste. This is a great pity, for the subject deserved better. □

John J. Videler is a Senior Lecturer in the Department of Zoology, Groningen State University, The Netherlands.

Catch control

D.H. Cushing

Fish Stock Assessment: A Manual of Basic Methods.

By J.A. Gulland.

Wiley: 1983. Pp.236. £15.95, \$31.95.

JOHN Gulland is a statistician with great experience of the needs of fish stock managers; he is as familiar with computer programs as with the envelopes on which he did essential sums for two International Fisheries Commissions assessing fish stocks.

His discussion of the data required, on catch per unit of effort, on average catchability, fishing effort, fishing power and fishing intensity is the work of one with very great experience. There are good accounts on the problems in handling the material for production and analytic models, not so much in statistical arithmetic but in the approach to judgment. The manual is intended for tyros but the old hands should read it carefully for the insights revealed from sentence to sentence.

The stages in the development of a fishery are described and they show why assessment is needed. A most useful section on the maximum sustainable yield shows how the concept remains valuable although for statistical and economic reasons, yields short of that maximum are desirable.

There is an interesting account of the unit stock, a difficult concept because it may be a species (as in the Southern Bluefin tuna), a subpopulation or even a group of species (as in the groundfish of the Gulf of Thailand). This is because there are operational requirements as well as the more obvious biological bases; I would have laid a little more stress on the genetic evidence. Current problems of density dependence and of multispecies interactions are stated and possible lines of research indicated.

There is a fisheries science (including studies of migration, bioenergetics, stabilization of populations amongst others) broader than that of stock assessment. But Gulland's manual will be of great value to assessment biologists with the responsibility for giving advice each year to the managers. □

D.H. Cushing is a retired fisheries biologist interested in fisheries science and stock assessment.

Refined analytical methods

Peter Brookes

Handbook of Polycyclic Aromatic Hydrocarbons.

Edited by Alf Bjørseth.

Marcel Dekker; 1983. Pp.744. \$150.

TO THE organic chemist or student of chemical carcinogenesis the words polycyclic aromatic hydrocarbons conjure up visions of complex synthetic methods, many and varied metabolic pathways, intricate reactions with cellular macromolecules and potent biological effects both *in vitro* and *in vivo*. The present book mentions none of these things. What therefore is left on the subject to fill a book of over 700 pages? To seek an answer one would need to consult physical or analytical chemists, industrialists or environmentalists, for whom this volume is primarily intended as a source of detailed information on the wide range of techniques applicable to the qualitative and quantitative analysis of these compounds.

In this respect, the Handbook has no equal. The chapters on the use of high-pressure liquid chromatography and spectrometric methods are particularly detailed and each is supplemented by about 200 references to the original literature. Gas chromatography/mass spectrometry are ideal analytical techniques and their power is well illustrated by application to coal-derived products. A single column run on the polycyclic aromatic hydrocarbon fraction of solvent-refined coal enables about 100 compounds to be identified and their concentration estimated.

The ubiquitous distribution of polycyclic aromatic hydrocarbons in the environment as a result of fossil fuel combustion, and the potent carcinogenicity of several members of the series, makes it desirable to measure their concentration in the air, soil, sea, lakes and rivers. Since all food stuff must be in contact with one of these sources, the levels of these hydrocarbons in food are of some concern, and these natural levels may be increased by cooking and preserving. Surprisingly, although malting of barley involves exposure to the smoke from peat, the levels of polycyclic aromatic hydrocarbons found in whisky are extremely low.

The Handbook is nicely produced and has much of its information presented in clear tables and figures. Few people would wish to read the whole book but many more would find it a useful reference volume for facts, and at least an introduction to methodology. □

Dr Peter Brookes is Reader in Chemistry of the University of London and Chairman of the Chemical Carcinogenesis Section, Institute of Cancer Research.

Biochemical instrumentation

This week's selection covers instruments for the general biochemical laboratory, including a microprocessor controlled dispensing system and a new power supply for electrophoresis.

Chromatography

● LDC/Milton Roy has produced a new pressure monitor, an accessory for HPLC components. The pressure monitor was designed to sense pressure in metering pumps that do not have an integral pressure transducer. It will interrupt the power, thereby shutting down the metering pump, if either the preset high or low limit is exceeded. The pressure monitor will also control and shut down any HPLC component with a 115 V, 10.0 A signal. High and low pressure limits can be set in either of 2 ranges: 0–6,000 p.s.i. for standard HPLC metering pumps and 0–12,000 p.s.i. for high pressure rated metering pumps.

Circle No. 100 on Reader Service Card.

● A new line of laboratory columns for medium-pressure liquid chromatography has been produced by Amicon. The new selection of standard columns offers pressure ratings from 45 to 100 p.s.i. for use with standard, as well as more rigid, gels. Columns can be either adjustable or fixed, jacketed or unjacketed.

Circle No. 101 on Reader Service Card.

● A new HPLC UV/visible absorbance detector, designed to be used with the full range of chromatographic equipment, has been introduced by Waters Associates. The Lambda-Max model 481 absorbance detector features a continuously variable wavelength range of 190–700 nm and full scale sensitivity settings of 0.0001–11.0 absorbance units. Three modular flow cells, packaged as plug-in modules, are available for microbore, analytical and gram scale preparative chromatography respectively. The Waters model 481 monitors sample absorbance, solvent purity, the condition of the lamp and the flow cell, and electronic performance. The light source is an aperture-type deuterium lamp whose electrode structure confines the light output to a 1-mm opening.

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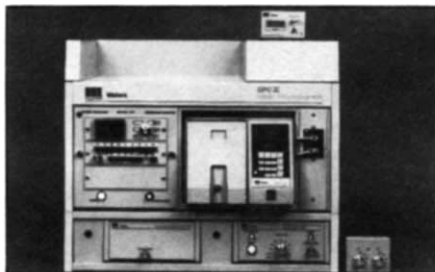
These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● A new series of integrated systems for performing gel permeation chromatography at ambient or elevated temperatures has been announced by Waters Associates. Waters GPC I and GPC II systems have been designed for the analysis of polymers or small molecules. The GPC I system detects differential refractive indices and GPC II incorporates Waters' model 441 UV/visible absorbance detector, with 229 nm as the standard wavelength. This wavelength is particularly well suited to the analysis of epoxies, but wavelengths from 214 to 658 nm can be selected for other applications. Both systems incorporate column temperature control from ambient to 120°C.

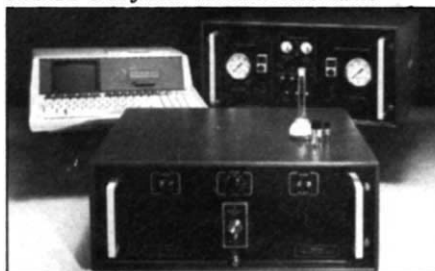
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● Reeve Analytical has introduced a radioactivity detector for HPLC. The detector weighs 23 kg and has a dynamic range of one part in 4×10^6 which is accessible to a minicomputer through an integral IEEE-488 instrument bus. Software support is available for data acquisition, storage, peak integration and the formatting of results by an HP-85 computer. A range of homogeneous and heterogeneous flow cells are available, the latter being packed with either cerium-activated lithium glass or yttrium silicate.

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The GPC II from Waters Associates



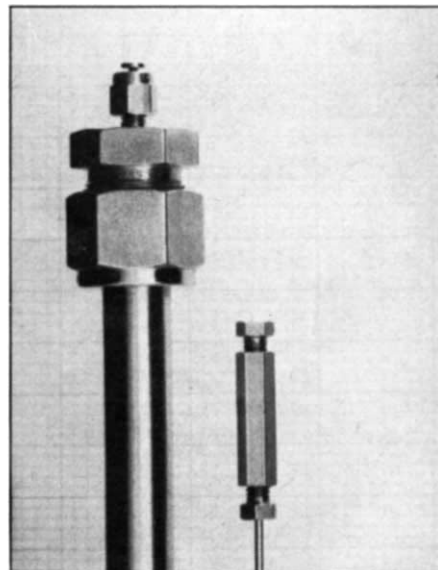
Reeve Analytical's radioactivity detector

● To make it easier to pack chromatography columns, Shandon have introduced additional slurry reservoir adaptors for their HPLC packing pump. The new adaptors are available for 3/8-inch Swagelok columns, Valco-type columns, and Water Associates columns (internal diameters: 4 and 7 mm). Using the adaptors, each type of column can now be connected to the Shandon slurry reservoirs (capacities: 12, 33 or 63 ml) enabling a range of columns to be packed. The existing standard 1/4-inch Swagelok slurry adaptor will continue to be available for use with the Shandon packing pump system. The packing pump itself requires neither pipework nor additional fittings, other than a suitable air supply. It operates up to a maximum pressure of 9,000 p.s.i., sufficient to pack all types of HPLC column in both 5 mm and 8 mm sizes.

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● A series of chiral bonded phase HPLC columns has been developed by ES Industries that will increase the scope of separations that can be achieved with pirkle columns. ES Industries chiral columns are applicable to both normal and reverse phase modes of separation. They are available in a variety of analytical and preparative sizes.

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HPLC columns from ES Industries

General laboratory equipment

● Burkard Scientific has introduced the PAX 100 dispensing system. The PAX 100 provides microprocessor-controlled aliquot volumes from 0.1 μ l to 99 μ l. Liquid metering in microlitres is set by a thumbwheel switch and the number of aliquots dispensed is indicated on a 4-digit 7-segment LED display. The two rates of dispensing for different viscosity ratings are confirmed by a panel indicator lamp. The dispenser uses "lock-out" circuitry to avoid double dispensing during delivery, and can be operated by a foot switch. The dispensing module is interfaced with the programmer through a 1-metre cable and 15-way D-type connector. This allows remote operation of PAX 100, for example in a safety cabinet. Additional extension leads are also available.

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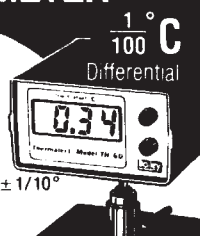
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● New from Demco is a micro-stirrer which is made to fit into the pH electrode holder on the electrode stand. The micro-stirrer can be used in pH titration, dissolved oxygen meters or in any situation where space is restricted, such as in test tubes where ordinary stirrers cannot be used. The micro-stirrer can be used with either a battery or a variable speed transformer.

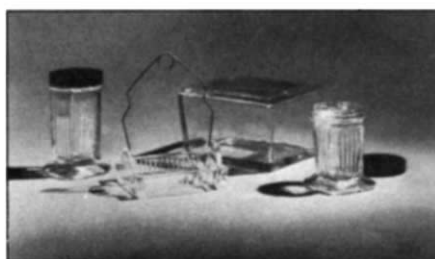
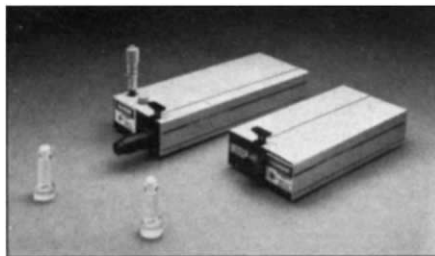
Circle No. 108 on Reader Service Card.

● Wheaton Scientific now offers both horizontal and vertical staining dishes capable of holding up to forty 3×1-inch (75×25-mm) microscope slides. These glass staining dishes clean easily and do not absorb stains, as do plastic dishes. The Wheaton Coplin staining jar holds five single slides and can also be used in thin-layer chromatography.

Circle No. 109 on Reader Service Card.

● Laser Science has introduced the first in a series of portable nitrogen and nitrogen laser pumped dye lasers, the VSL-337 and the VSL-Dye, both designed for use as laser light sources in biochemical instruments. The VSL-337 is a pulsed nitrogen laser in the near UV range at 337 nm, with a peak power of 40 kW and a pulse length of 3 ns. The repetition rate is 0–20 p.p.s. The VSL-337 has a disposable integrated sealed plasma chamber, with a lifetime of 10⁷ pulses. This means that all the active components with inherent finite lifetimes are located in a single disposable cartridge that can be replaced. The VSL-Dye combines a VSL-337 nitrogen laser and a grating-tunable dye laser with an output in the visible range of the spectrum from 400–800 nm and a direct wavelength readout. The peak power is 5 kW. Uses of the VSL lasers include microspectrophotometry and microdensitometry.

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Staining dishes from Wheaton Scientific

● The new Spectroflow 400 solvent delivery system is available from Kratos. Flow range on the Spectroflow 400 is 10 μ l per minute to 5 ml per minute, which makes the instrument suitable for microbore liquid chromatography, fast liquid chromatography, and conventional HPLC. It is not necessary to change pump heads or electronics on the Spectroflow 400 to operate it at microbore flow rates. Accuracy is $\pm$ 0.1% of setting. The Spectroflow 400 can be operated at pressures up to 7,000 p.s.i. Front panel controls permit the selection of upper and lower pressure limits, and the system has purging capabilities. An optional binary or ternary gradient former can be added without the need for additional pumps.

Circle No. 111 on Reader Service Card.

● A series of pH and redox probes suitable for use at high pressures (to 10 bar) and high temperatures (to 130°C) in reaction vessels, fermentation vats and pipelines, is now available from Tekmar. Tekmar select which of their electrodes, of lengths from 50 to 500 mm, is most suitable for each use on the basis of a sample furnished by the customer. All fluid-wetted portions are made of stainless steel and the rest of the probe is made of nickel-plated brass, except for the head, which is polypropylene.

Circle No. 112 on Reader Service Card.

● A new batch sampler, the model 3000, has been released by Hiac/Royco Instruments Division. The sampler has a digital encoder attached to the syringe plunger that converts its position to a digital signal so that the microprocessor can control sample volume to within 1% and flow rate to within 5%. The product features digitally-selectable sample volumes of 0.1 to 10 ml or 1 to 100 ml and a flow rate that is independent of sample viscosity.

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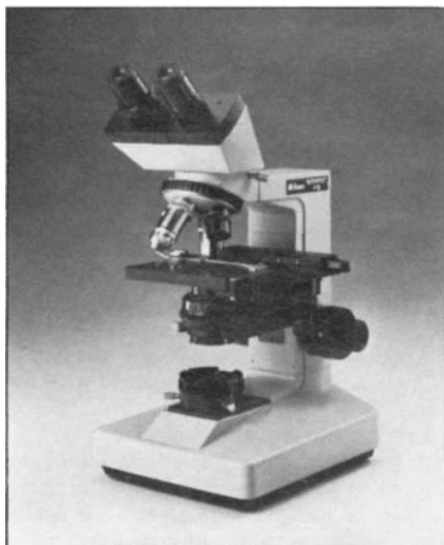
Hiac/Royco's new batch sampler

● The ECPS 3000/150 from Pharmacia Fine Chemicals is a high voltage power supply with constant voltage, constant current or constant power regulation. Crossover from one mode of control to another is automatic, and the high voltage output makes the instrument well suited for use in DNA sequencing and isoelectric focusing (IEF). A range switch (15W/150W) ensures improved accuracy when the power supply is used in high voltage and low power applications such as ultrathin IEF. An optional accessory is the Volt-hour integrator VH-1, which accurately measures the extent of an electrophoresis experiment in volt-hours.
Circle No. 130 on Reader Service Card.

● Spectra-Physics has announced that the SP4270 chromatography integrator is now available with an optional CRT terminal. The 12-inch diagonal screen has a format of 24 lines by 80 characters per line. This CRT terminal allows the operator to set or modify any file in the SP4270, running at transfer rates up to 2,400 Baud. BASIC programs can be written for the SP4270 with the detachable keyboard, which is also equipped with a separate numeric keypad. Results can be automatically displayed on the CRT at the end of each run.
Circle No. 114 on Reader Service Card.

● Harvard Apparatus now offer a new syringe pump designed to hold either 50-ml or 30-ml disposable plastic syringes. Each syringe offers a total of 999 discrete flow rates which can be selected from 99.0 ml per hour down to 00.1 ml per hour.
Circle No. 115 on Reader Service Card.

● Nikon has just introduced Alphaphot, the new microscope from the YS-series for use in the laboratory and classroom. Alphaphot is compatible with Nikon's microscope accessories, including an epifluorescence attachment, an epifluorescence illuminator and a phase contrast system.
Circle No. 116 on Reader Service Card.



The Alphaphot microscope from Nikon

● Brinkmann Instruments has announced a new addition to its line of liquid handlers: the Brinkmann range of micro-dispensers. The dispensers are designed for repetitively dispensing microlitre volumes directly from small vials and bottles. They are available in both fixed and adjustable volume models. Fixed volume models are calibrated in the factory. Adjustable volume models can be set and the desired volume locked into place to avoid accidental volume change.

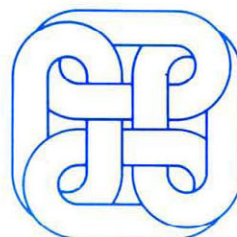
Circle No. 117 on Reader Service Card.

● Gripsafe beaker tongs for lifting hot or cold containers from hot-plates or cold freezers have been introduced by Chemtrix. These steel tongs will hold most glassware, pots or pans and have a built-in bottle cap remover and a hole for hanging.
Circle No. 118 on Reader Service Card.

● A range of instruments designed to automate the techniques of cellular engineering, as distinct from genetic engineering, are now available from Endotronics. The cellular engineering technology communicates with cells, tissues and small organs by controlling the biochemical environment around them. The instruments, called Acusyst, regulate tissue function *in vitro* by controlling the delivery of regulatory agents to isolated tissues.
Circle No. 119 on Reader Service Card.

● The bench-top Delta 300 liquid scintillation system is now available from Tracor Analytic with the new BetaComp data processor. BetaComp is a data reduction package that automatically calculates the dose per minute for single and dual label studies.

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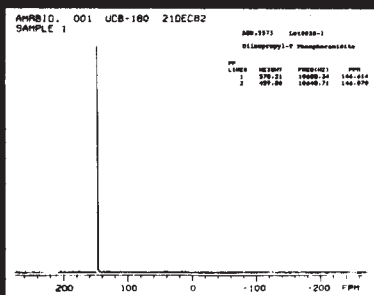
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Analysers

● The new model KN-03 Kjeldahl nitrogen analyser is capable of measuring the Kjeldahl nitrogen content of foods, biological specimens, fertilizers and foodstuffs. To operate the system, sample volumes of 10 to 200 µl Kjeldahl digestion solution are injected into the analyser, which then neutralizes, distills and titrates them. It then calculates and displays the results digitally. Ammonium ions in the injected sample contact hot alkali to liberate ammonia. This is then dehydrated and transferred to the titration cell by a carrier gas, where it is then automatically titrated by coulometry. This operation takes 5–10 minutes. The measuring range of the instrument is 0.1 to 19.99 µg nitrogen. For a typical 100 measurements, 50 ml of 1% Na₂SO₄ electrolysis solution and 50 ml of KOH solution are required. *Circle No. 121 on Reader Service Card.*

New literature

● A new assay reference guide that features a topical index for choosing appropriate drug screening tests is being offered by Biomeasure. The guide also explains the 32 assays that are performed by Biomeasure. The guide lists receptor binding, enzyme and cellular assays, and animal tests to evaluate the biological interactions and pharmacological effects of research substances. The guide details assay concepts and methodologies, cellular interactions, physiological functions and typical results. Also listed are the enzyme sources, ligands and agents that are used in specific assays. *Circle No. 124 on Reader Service Card.*

● A full-colour brochure has been released by Orion that describes their model 1020 sodium/potassium clinical analyser. The new analyser features a 16-character alphanumeric display on which the results are shown 50 seconds after a 100-µl sample of whole blood, serum or plasma has been aspirated. A 400-µl sample of diluted urine takes 65 seconds to analyse. A complete cycle includes sample measurement, automatic flushing and a one-point calibration. *Circle No. 125 on Reader Service Card.*

● J.T. Baker Chemical Company has published the Baker-10 SPE applications guide, volume I. This is a guide to preparing samples for the Baker-10 SPE system. It highlights the background, principles and techniques of solid phase extraction (SPE) and directions are given for SPE column selection, conditioning, sample addition, washing and elution procedures. The guide contains procedures for preparing environmental, pharmaceutical, biological and cosmetic samples. *Circle No. 126 on Reader Service Card.*

● Anachem has brought out a new sulphur analyser. The model TS-02 sulphur analyser uses oxidative pyrolysis followed by coulometric titration to investigate samples. The dynamic range of the TS-02 is from 0.1 to 2,000 p.p.m. This makes the analyser suitable for performing both trace level analyses and high sulphur concentration determinations without dilution. *Circle No. 122 on Reader Service Card.*

● A new chemistry analyser has been produced by Baker Instruments. The series 1-2-3 chemistry analyser details each analytical procedure from reagent preparation to printed results. The removable program module automatically sets and controls all functions, including wavelength selection and temperature setting. Wavelength selection is achieved by a constantly spinning filter wheel that enables the microprocessor to read continuously as the proper filter is in line. *Circle No. 123 on Reader Service Card.*

● LKB has developed the first of a series of biotechnology brochures designed to highlight a phase in the process of generating and isolating biomolecules. The initial brochure is intended to serve as a guide to the use of high-resolution separation techniques in the secondary purification stage. The brochure details some of the innovative products and methodologies offered by LKB and outlines their applications in secondary purification processes. Included are media for gel filtration, ion exchange, affinity, adsorption and high performance liquid chromatography. *Circle No. 127 on Reader Service Card.*

● A 32-page catalogue on glass distillation systems, components and control accessories is now available from H.S. Martin. The brochure features the perforated plate, the bubble cap, the packed distillation columns and a complete line of industrial coil condensers, reactors and heat exchangers. Data are also provided on spinning band stills, adiabatic columns and completely automated systems. *Circle No. 128 on Reader Service Card.*

● A catalogue from New Brunswick Scientific describes 19 biological shakers with gyrotory and reciprocal drives for bench-top, warm room, controlled temperature and industrial applications. Illustrated in the catalogue are small, portable shakers, water bath and controlled environment incubator shakers for laboratory applications and multitiered large volume industrial shakers. Controlled environment models with top and front loading chambers provide ± 0.5°C temperature regulation over the range 0–60°C and adjustable shaking rates of 40 to 400 r.p.m. A selection of interchangeable accessory platforms is available for flasks, test tubes and beakers. *Circle No. 129 on Reader Service Card.*